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Aims and Scope

IUFS Journal of Biology is a semi-annual, international, peer-reviewed journal in English and publishes original research articles, reviews and short communications in the field of biological sciences. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

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Research articles

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Research Article

The changes of oxidative stress and Δ^9-tetrahydrocannabinol accumulation in liver of type-2 diabetic rats

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Abstract

In this study, we aimed to explore the changes of oxidative stress, Δ^9- tetrahydrocannabinol (Δ^9-THC) and its metabolites accumulation in the liver of type-2 diabetic rats treated with Δ^9-THC. 8-10 week-old Sprague-Dawley rats were divided into four groups. Group I: Physiological saline was administered intraperitoneally(i.p) (n=7). Group II: Rats that was given Δ^9-THC for 7 days (3 mg/kg/day) (n=6) (i.p). Group III: Streptozotocin(STZ, 65 mg/kg)+Nicotinamide(NAD, 85 mg/kg) (n=7) (i.p). Group IV: Diabetic rats that were given Δ^9-THC(3 mg/kg/day) for 7 days (n=7) (i.p). The biochemical investigation was carried out on the serum and liver tissue. Δ^9-THC and its metabolites were analyzed by using GC-MS in the liver of rats. Liver glutathione level in diabetes+ Δ^9-THC increased as compared to diabetic rats. The increased lipid peroxidation and protein carbonyl levels in diabetes showed a low reduction with Δ^9-THC. Catalase and superoxide dismutase activities in liver of diabetic rats increased with Δ^9-THC treatment, non-statistically. Serum uric acid level, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities slightly increased in diabetic group as compared to control group. While the AST level reduced in the liver, ALT level did not be affected in Δ^9-THC treated with diabetic rats as compared with the diabetic group. Δ^9-THC level in liver was negative in given Δ^9-THC groups. But the liver THC-COOH metabolite accumulation in Δ^9-THC group is higher than Diabetes + Δ^9-THC group. We could say that Δ^9-THC can reduce diabetes-induced oxidative damage in the liver of type-2 diabetic rats. The metabolization of Δ^9-THC in the liver can be accelerated with diabetes.

Keywords: Type-2 diabetes, Δ^9-tetrahydrocannabinol, liver, oxidative stress, rat
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Tip-2 diyabetik sıçanların karaciğer dokusundaki oksidatif stres ve Δ^9- tetrahidrokannabinol birikim değişiklikleri

Özet

Çalışmamızda, Δ^9-THC uygulanan tip-2 diyabetik sıçanların karaciğerlerinde oksidatif stres ve Δ^9-THC birikim değişikliklerinin incelenmesi amaçlanmıştır. 8-10 haftalık, erkek Sprague-Dawley sıçanlar dört gruba ayrıldı. Grup I: Serum fizyolojik intraperitoneal (i.p.) olarak uygulandı (n=7). Grup II: Sıçanlara 7 gün süresince Δ^9-THC (3 mg/kg/gün) (i.p.) verildi (n=6). Grup III: Streptozotosin (STZ, 65 mg/kg) + Nikotinamid (NAD, 85 mg/kg) (n=7) (i.p.).Grup IV: Diyabetik sıçanlara 7 gün Δ^9-THC (3 mg/kg/gün) (i.p.) (n=7) verildi. Serum ve karaciğer dokularında biyokimyasal incelemeler yapıldı. Karaciğerde Δ^9-THC ve metabolitleri GC-MS kullanılarak analiz edildi. Diyabet + Δ^9-THC grubunda karaciğer glutatyon seviyesi diyabetik sıçanlara göre arttı. Diyabette artmış olan lipid peroksidasyon ve protein karbonil seviyeleri Δ^9-THC uygulamasıyla düşük seviyede bir azalma gösterdi. Diyabetik sıçanların karaciğerlerinde katalaz ve superoksid dismutaz aktiviteleri Δ^9-THC uygulamasıyla istatiksel olarak anlamsız olarak arttı. Kontrol grup ile kıyaslandığında, serum ürik asit seviyeleri alanin aminotransaminaz (ALT) ve aspartat aminotransaminaz (AST) aktiviteleri, diyabetik grupta çok az arttı. Diyabetik grup ile kıyaslandığında, diyabetiklere Δ^9-THC uygulaması karaciğerde AST'yi düşürüken ALT'yi etkilemedi. Δ^9-THC verilen grupta karaciğer Δ^9-THC seviyesi negatifti. Fakat Δ^9-THC grubunda karaciğer THC-COOH metabolit birikimi diyabet + Δ^9-THC grup ile kıyaslandığında daha yüksekti. Tip 2 diyabetik sıçanlara Δ^9-THC uygulanmasının, karaciğerde diyabetin neden olduğu oksidatif hasarı azaltabileceğini söyleyebiliriz. Δ^9-THC'nin karaciğerde metabolizasyonu diyabet ile hızlanabilir.

Anahtar kelimeler: Tip-2 diyabet, Δ^9-tetrahidrokannabinol, karaciğer, oksidatif stres, sıçan

Introduction

It has been known that cannabinoids were isolated from the cannabis plant (*Cannabis sativa* L.) and Δ^9-tetrahydrocannabinol (Δ^9-THC) is the main active psychoactive constituent of *C. sativa* (Kochanowski and Kala 2005; Ahmed et al. 2015). Δ^9-THC turn into its active metabolite 11-hydroxy-Δ^9-THC (11-OH-THC), and then converted to an inactive metabolite 11-nor-9-carboxy- Δ^9-THC (THC-COOH) (Kochanowski and Kala 2005; Gieringer et al. 2008). Δ^9-THC and its metabolites may be determined in blood, liver and kidney tissues (Kemp et al. 2015). It is reported that *C. sativa* and its products have been used as medicinal agents (Elsohly and Slade 2005). According to Fasinu et al. (2016), sixteen states in the United States have legalized cannabidiol (CBD) and Δ^9-THC contenting products due to using in medicine. Pinto et al. (2010) reported that Δ^9-THC administration has not any harmful effects on healthy mice liver. Furthermore, according to Chen et al. (2005), Δ^9-THC can block reactive oxygen radicals (ROS) generation.

Type 2 diabetes is the most common type of *Diabetes mellitus* which is a multifactorial disease and characterized by hyperglycemia and insulin resistance (Verspohl 2009). Oxidative stress is based on an imbalance between antioxidants and the generation of ROS (Kassab and Piwowar 2012). The studies have shown that oxidative stress plays an important role in diabetes (Baburao and Anand 2012; Stadler, 2012; Ávila Dde et al. 2013).

The aims of the present study; (i) to explain the effects of Δ^9-THC on oxidative stress in liver of type 2 diabetes, and (ii) to detect the level of Δ^9-THC metabolites in the liver of diabetic and control animals treated with Δ^9-THC.

Materials and methods

Animals and ethics

8–10 weeks-old male Sprague-Dawley rats were housed in a temperature-controlled clean room with a 12 h light/dark cycle and fed with tap water and standard chow *ad libitum*. All experimental procedures were performed according to the guidelines of the Local Ethic Committee of Animal Research (Istanbul University, HADYEK 2015/06).

Experimental design

The animals were selected randomly and arranged into four groups;

Group 1: (n = 7) Control rats that received serum physiologic intraperitoneally (i.p.).

Group 2: (n = 6) Δ^9-THC (3 mg/kg/day, i.p. Lipomed THC-135-100LE) was administered to the rats for seven days.

Group 3: (n = 7) For diabetes, the rats were injected with a single dose of streptozotocin (STZ) (65 mg/kg, Sigma-Aldrich S0130) dissolved in serum physiologic 15 min after the injection of nicotinamide (NAD) (85 mg/kg, Sigma-Aldrich N3376) in serum physiologic (i.p.) (Masiello et al. 1998).

Blood glucose levels of the rats were measured 72 h after the STZ + NAD injection. The animals with blood glucose concentrations more than 200 mg/dL were accepted as diabetic.

Group 4: (n = 7) diabetic animals were treated (i.p.) with Δ^9-THC (3 mg/kg/day) during 7 days.

The experiment was terminated on day 15th after the Δ^9-THC injections, the animals were anesthetized i.p. with ketamine-HCl (50 mg/kg, Pfizer) and xylazine-HCl (10 mg/kg, Bayer). Immediately, the blood and liver tissue samples were collected from rats.

Analysis of serum and liver tissue

The blood samples were centrifuged, and serums were separated. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and uric acid (UA) level were measured in all serum samples using the Siemens Advia 1800 chemistry system. The liver tissue samples were homogenized in cold 0.9 % NaCl and made up to 10 % homogenate. The homogenates were centrifuged. The clear supernatant fractions were removed for the biochemical analysis. The protein content was determined by Lowry's method using bovine serum albumin as standard (Lowry et al. 1951). The glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels in liver homogenates were estimated according to Beutler's, Ledwozyw's and Reznick and Packer's methods, respectively (Beutler 1963; Ledwozyw et al. 1986; Reznick and Packer 1994). Liver catalase (CAT) activity was determined by using Aebi's method (Aebi 1984). The superoxide dismutase (SOD) activity was assayed in liver tissues by using the method of Sun (Sun et al. 1988). Δ^9-THC and its metabolites were analyzed by using GC-MS in the liver of rats.

Statistical analysis

The statistical analysis was conducted using the SPSS 21.0 software program. The descriptive statistics of the data were expressed using the mean ± standard error of the mean (SEM). The data were evaluated for statistical significance using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

Results

The levels of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and uric acid (UA) are seen in Fig.1. ALT and AST levels showed an insignificant increase in type 2 diabetic rats as compared to control group. It was determined that while serum ALT level did not show any change in Δ^9-THC treated with diabetic rats as compared to the diabetic group, AST level decreased, non-significantly. A slight increase in serum UA level was observed in STZ/NAD-induced rats when compared to control group. The increased UA level in diabetic was reversed with Δ^9-THC treatment, insignificantly. In Table 1, Liver glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels are presented. The liver GSH levels insignificantly decreased in diabetic group as compared with control rats. GSH levels increased by treatment with Δ^9-THC in diabetic rats compared to the diabetic group.

Δ⁹-THC administration to diabetic reduced LPO and PCO levels in the liver as compared to non-treated diabetic rats. The liver catalase (CAT) and superoxide dismutase (SOD) activities in diabetic rats reduced when compared with control rats. Both of enzyme activities showed an insignificant increase in STZ/NAD-induced diabetic animals with Δ⁹-THC treatment (Table 2). We examined the accumulation of Δ⁹-THC and its metabolites in liver tissues of Δ⁹-THC and diabetes + Δ⁹-THC groups. Δ⁹-THC level in liver was negative in given Δ⁹-THC groups. But the liver THC-COOH metabolite accumulation in Δ⁹-THC group is higher than STZ/NAD-induced diabetes + Δ⁹-THC group, non-significantly (Fig. 2).

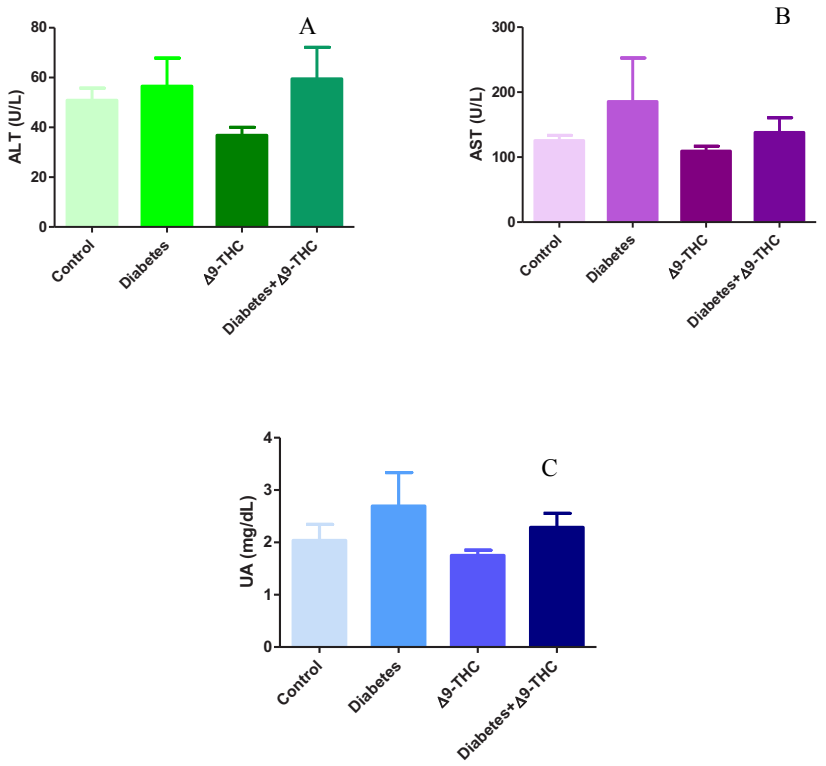

Figure 1. The levels of liver enzymes and uric acid in serum. A: alanine aminotransferase (ALT); B: aspartate aminotransferase (AST) and uric acid (UA). Data are expressed as mean ± SEM.

Table 1. Liver glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels in all groups.

	GSH (nmol/mg protein)*	LPO (nmol/mg protein)*	PCO (nmol/mg protein)*
Control	7.36 ± 0.61	0.14 ± 0.03	0.36 ± 0.07
Diabetes	6.05 ± 0.31	0.21 ± 0.02	0.56 ± 0.17
Δ⁹-THC	6.53 ± 0.29	0.18 ± 0.02	0.32 ± 0.06
Diabetes + Δ⁹-THC	6.88 ± 1.25	0.19 ± 0.01	0.44 ± 0.11
P_{ANOVA}	> 0.05	> 0.05	> 0.05

*Mean ± standard error (SEM)

Table 2. The catalase (CAT) and superoxide dismutase (SOD) activities in liver of rats.

	CAT (U/mg protein)*	SOD (U/mg protein)*
Control	92.62 ± 16.7	1.30 ± 0.11
Diabetes	5.70 ± 2.67	1.19 ± 0.03
Δ^9-THC	54.71 ± 24.56	1.04 ± 0.02
Diabetes + Δ^9-THC	67.34 ± 31.79	1.94 ± 0.63
P_{ANOVA}	> 0.05	> 0.05

*Mean ± standard error (SEM).

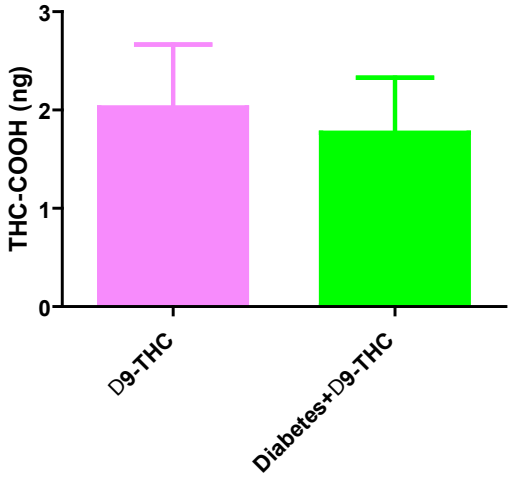

Figure 2. The levels of THC-COOH (ng) in liver of Δ^9-THC and STZ/NAD-induced diabetes + Δ^9-THC groups on day 15 after the Δ^9-THC injections. Data are expressed as mean ± SEM.

Discussion

The present study shows that STZ/NAD-induced type 2 diabetes caused the rise of oxidative damage in the liver of rats. Furthermore, treatment with Δ^9-THC modulated STZ/NAD-induced biochemical changes. The accumulation of Δ^9-THC was detected in the liver of rats.

Type 2 diabetes causes insulin resistance and decreased insulin secretion. The disease progression induced tissue damages shows various symptoms of complications (Malenica et al. 2016). The liver is an important organ in the regulation of glucose level. The clinical and experimental studies reported that liver cell damage occurs in type 2 diabetes (Parveen et al. 2010; Ramljak et al. 2015). There are strong evidence that increased oxidative stress is related with the pathogenesis of many diseases. Moreover, oxidative stress plays also a major role in the development of diabetes complications (Oliveira et al. 2016; Salmon 2016). In the previous study, LPO and PCO levels increased in liver, small intestine and stomach of type 2 diabetic neonatal rats and GSH level decreased (Karatug et al. 2012; Coskun et al. 2013; Koyuturk et al. 2015). Likely, we observed similar changes in LPO, PCO and GSH levels in the liver of diabetic adult rats. Individuals with type 2 diabetes and experimental diabetic rats have rising liver enzymes levels in serum as compared to controls (Harris 2005; Koyuturk et al. 2015). Similar to previous studies, we found the serum ALT and AST liver enzymes raised in STZ/NAD- induced diabetes group when compared with healthy rats.

Investigators noticed medical plants can use against oxidative damage in experimental diabetic rats (Oliveira et al. 2016). Recently, pharmacological studies have been carried out with the therapeutic properties of Δ^9-THC obtained from *C. sativa* plant. Chen et al. suggested that Δ^9-THC should play a role as an antioxidant substance (Chen et al. 2005; Mcpartland et al. 2015). In the present study, we applied non-synthetic Δ^9-THC to STZ/NAD-induced rats. Δ^9-THC treatment changed the oxidative stress parameters as non-significantly.

A modulation was observed oxidative stress state in diabetic rats treated with Δ^9-THC. The reduction in activities of enzymatic antioxidants such CAT and SOD is caused the elevation of ROS. Arulselvan and Subramanian (2007) reported that the increased oxidative stress in diabetes might be induced a decrease of CAT enzyme activity in tissue. Furthermore, it was observed that SOD enzyme activity in diabetic rats reduced (Sozmen et al. 2001). In this study, both enzymes showed a reduction in diabetics, but Δ^9-THC administration elevated the antioxidant enzymes to some extent on diabetic animals. Δ^9-THC may decrease oxidative stress sin diabetes by raised antioxidant enzymes.

Δ^9-THC accumulates been observed in adipose and brain tissues (Kreuz and Axelrod 1973). Addition to, profiles of Δ^9 -THC and its metabolite can be detected in plasma samples (Brenneisen et al. 2010). The excretion of Δ^9 -THC and its metabolite 11-OH-THC were measured in urine of chronic cannabis users for -4, -7 and -24 days after the cannabis cessation. Its metabolites were detectable in urine at lowest levels in 24 days (Lowe and Axelrod 2009).

According to our previous study, the Δ^9-THC metabolite level in urine of non-diabetic rats was lower than that of the diabetic animals. Otherwise, the accumulation of THC-COOH in the liver of non-diabetics was higher than of the diabetic rats. It pointed out that liver may lay up Δ^9-THC and its metabolite, their excretion with urine notwithstanding.

Conclusions

Our findings indicate that Δ^9-THC administration may serve a protective role to some extent for liver in diabetes by blocking oxidative stress state. The metabolization of Δ^9-THC in the liver can probably be accelerated with diabetes. However, further investigation is necessary to clarify the effects of Δ^9-THC supplementation on type 2 diabetes.

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Research Article

Investigation of chromosome variation in four *Aegilops* L. (Poaceae) species and populations in Iran

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Abstract

The genus *Aegilops* is one of the most important genera of the Poaceae family. It belongs to the tribe Triticeae with 12 species in Iran. The research karyological analyzed in 12 populations of 4 *Aegilops* species: *A. umbellulata* Zhuk., *A. tauschii* Coss., *A. columnaris* Zhuk. and, *A. triuncialis* L. were done. We used fresh grown root tips. Then α-bromonaphtaline, formaldehyde and chromium trioxide (1:1), 1 N NaOH and hematoxiline were used for pre- treatment, fixative, hydrolyser and chromosome staining agent, respectively. We used video Analysis system for each species with Micromeasure software. All populations in the secondary basic numbers were x=7. The ploidy levels were different. In *A. tauschii* and *A. umbellulata* were found 2n=14, and *A. columnaris* and *A. triuncialis* were found 2n=28. Detailed karyotype analysis allows us to group the different species and to postulate relationships among them.

Keywords: *Aegilops*, Chromosome, Karyology, Poaceae, Populations
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Introduction

The genus *Aegilops* L. (Poaceae) is one of the wheat relatives with a wide distribution in Iran, and is capable of making different complexes with each other and with *Triticum* L. genus. It has 12 species in Iran (Boissier 1844; Al- Mashhadani 1980). *Aegilops* is a western Asia-Mediterranean element found around the Mediterranean Sea and the western part of Asia (Bor 1968). The life form of Aegilops species is annual with a mainly outcrossing breeding system (Waines and Barnhart 1992). But some of the species such as *A. tauschii* is a self-pollinating (cleistogamic) goat grass species in the Triticeae tribe of the grass family. This genus is a pastoral plant that consists of only one donor species to the gene pool of *Triticum* L. (Lange and Jochemsen 1992a) and causes the evolution of hexaploid wheat (Waines and Barnhart 1992). *Aegilops tauschii* encompasses four morphological varieties, of which three, var. *typica*, var. *anathera*, and var. *meyeri*, are grouped taxonomically into *A. tauschii* ssp. *tauschii*, while the fourth is the monotypic *A. tauschii* ssp. *strangulata* (Eig 1929; Kihara and Tanaka 1958).

The ploidy levels of *Aegilops* species are diploid (2n = 2x = 14, x = 7), tetraploid (2n = 4x = 28, x = 7), and hexaploid (2n= 6x = 42, x = 7) (Bor 1968; Lange and Jochemsen 1992a, 1992b). All the diploid species have rather limited areas of distribution, while the tetraploid and hexaploid have a wider ecological adaptation (Hammer 1980). The *Aegilops* genus has played a major role in the constitution of durum and bread wheat genomes. It possesses wild species along with native races (Lange and Jochemsen 1992a,

1992b). Iran is one of the centers of distribution and variation of *Aegilops* in the world. Due to the importance of cultivated wheat, having a better knowledge about new genetic resources is necessary to improve wheat races.

Percival (1926) and Kihara (1937) studied the morphology and cytology of some hybrids of *Triticum* and *Aegilops*. Senyaninova (1932) recorded karyo-systematical investigation of *Aegilops*. Chennaveeraiah (1960) studied karyomorphologic and cytotaxonomic in *Aegilops*. Karataglis (1975) studied karyotype analysis on some diploid native Greek *Aegilops* species. Al-Mashhadani et al. (1980) recorded Karyotype analysis of five tetraploid *Aegilops* species native to Iraq. Badaeva et al. (1994) showed intraspecific karyotype divergence in *Triticum araraticum*. Ahmadabadi et al. (2005) recorded Intraspecific Karyotype Divergence of *Aegilops triuncialis* in the Northwest Regions of Iran. Results indicate the presence of high genetic variation among the populations of *A. triuncialis* species. This variation can be useful in breeding programs of polyploid wheat and to broaden the genetic variability of the gene pool. Karimzadeh et al. (2010) studied cytogenetic of some Iranian Wild Wheat Species (*Aegilops*) and OR-Banding.

Generally, taxa will cross more readily when brought to the same ploidy level. Exceptions, however, are encountered were cross ability at uneven levels is more successful. The 2n number alone, however, is of little use in determining species relationship. For that purpose, karyotype studies may provide more information. In addition, we need to examine chromosomal variation further due to morphological and physiological variation among the same *Aegilops* species collected in different places of the world (Kihara and Tanaka 1958).

Chromosome information is an important key for taxonomy, phylogeny, evolution, genetic and breeding in *Aegilops* plants. Here we present the report of the chromosome numbers, ploidy levels and comparison of karyotypic traits of some annual species and population of *Aegilops* genus in Iran. Our results are useful for a better understanding of its taxonomy and breeding purposes such as inter and intraspecific hybridization and genetic variation induction.

Materials and methods

The studied about 12 populations of 4 *Aegilops* species: *A. columnar*, *A. tauschii*, *A. triuncialis* and *A. umbellulata* with three populations for each species. The studied populations are listed in (Table 1). Vouchers deposited in the Herbarium of the natural research center of Fars province and in gene bank RIFR (Research Institute of Forest and Rangelands) from Iran. The mature seeds of four taxa were taken from the herbarium materials.

Table 1. The examined populations of *Aegilops* genus, Gene bank code, and location.

No.	Population	Gene bank code (RIFR)	Location
1.	*A. columnaris*	4134	Golestan: Minoodasht (1050m)
2.	*A. columnaris*	4135	Golestan: Minoodasht (1280m)
3.	*A. columnaris*	16500	Mazandaran: Larijan
4.	*A. tauschi*	13938	Golestan: Gonbad-kavoos
5.	*A. tauschi*	16379	Golestan: Tuska-stan
6.	*A. tauschi*	16426	Mazandaran: Alashet
7.	*A. triuncialis*	16418	Tehran: Abali
8.	*A. triuncialis*	16577	Fars: Fasa, Mianjangal
9.	*A. triuncialis*	15368	Fars: Shiraz, Hosain abad station
10.	*A.umbellulata*	15594	Fars: Fasa, Mianjangal
11.	*A. umbellulata*	8762	Fars: Shiraz, Hossein abad station
12.	*A. umbellulata*	3771	Fars: Darab, Layzagan

We used root tip meristems from seedling obtained by the germination of ripe seeds on wet filter paper in Petri dishes and left at 22°C temperature. Different pre-treatments were tested, and the best results were obtained for treating the root tips meristems with 0.5% saturated α-Bromo naphthalene at 4°C for 4-5 h, fixed in 10% formaldehyde and chromium trioxide (1:1 volume ratio) for 16 to 20 h at 4°C. Then the root tips were rinsed for 3 h in distilled water. Hydrolysis was carried out with 1 N NaOH at 60ºC for 20-25 min and used hematoxylin-iron for chromosome staining for 1-2 h at room temperature. Root tips were Squashed in a droplet of 45% acetic acid and lactic acid (10:1) (Wittmann 1965; Hesamzadeh Hejazi and Rasuli 2006). The preparations were observed with an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of about 2000x. Chromosomal recounts were done in at least five complete metaphases and used to prepare the karyotype by Adobe Photoshop 7.0 software and measured by Micro Measure 3.3 software for each genotype (Reeves and Tear 2000).

In each mitotic metaphase (at least 5 plates), cytogenetic parameters were calculated as: long arm (LA), short arm (SA), total length (TL), the percent of relative length of each chromosome (RL%), arm ratio (AR), centromeric index (CI), value of relative chromatin (VRC). Karyotype asymmetry was estimated by three different methods namely: total form percentage (TF%), a difference of relative length (DRL), intrachromosomal asymmetry index (A_1) and intrachromosomal asymmetry index (A_2).

Both indices A_1 and A_2 in (Romero-Zarco 1986) independent of chromosome number and size. Karyotype symmetry was determined according to Stebbins (SC) (Stebbins 1971). Chromosomes were identified according to Levan (Levan et al. 1964). For each species, karyograms and haploid idiograms were drawn based on the mean centromeric index and arranged in order of decreasing size. In order to determine the variation between species, one – way analysis of variance (ANOVA) was performed to compare the chromosomes pair in each population by Duncan's test. Factor analysis based on principal components analysis (PCA) was performed on standardized karyological data of populations. Cluster analysis using Ward's method was performed after calculation of Cophenetic correlation coefficient (*r*) to examine karyotype similarity among populations. Statistical analyses were performed using SAS ver. 6.12, 1996, JMP ver. 3.1.2, 1995 and Statisti XL ver 1.7, 2007 softwares.

Results

There was no different basis chromosome among the species (x=7). The somatic chromosome numbers (2n), karyotype formulae and parameters for the studied species are summarized in Table 2. Among 12 populations, 6 populations were diploid belong to *A. tauschii* and *A. umbellulata* and 6 populations were tetraploid belong to *A. columnaris*, *A. triuncialis* (Table 2). The pictures of the mitotic metaphases and their karyograms of the populations were presented in Fig. 1.

The mean value of chromosome's long arm was varied from 6.05 µm in *A. triuncialis* (16418) to 4.42 µm in *A. tauschii* (16379). Averages of chromosome's short arm were different from 3.84 µm in *A. tauschii* (16426) to 1.82 µm in *A. triuncialis* (15368). The total length of the chromosome was varied from 9.37 µm in *A. tauschii* (16426) to 6.72 µm in *A. triuncialis* (15368) and the mean value of chromosome's arm ratio was in the range from 2.75 in *A. triuncialis* (16418) to 1.38 in *A. tauschii* (13938) (Table 4).

In all of the population, the chromosomes were mainly of m (metacentric) type or sm (sub-meta centric) type. Satellites were observed in one chromosomes pair in *A. umbellulata* species and for two species *A. columnaris* and *A. triuncialis* which have two chromosomes pairs having satellites (Fig. 1).

Symmetry type of Stebbins (1971) and asymmetry indices of Romero-Zarco (1986) are given in (Table 2). According to of Stebbins category, *A. tauschii* species (13938 and 16379) populations were placed in 1A class, and *A. tauschii* species (16426) was placed in class 2A. *A. columnaris* species (4134, 4135 and 16500) populations were placed in 4A, 3B and 3A respectively. *A. triuncialis* species (16418, 16577 and 15368 populations) are classified as

4A, 3A and 4A category respectively. Finally, *A. umbellulata* species (15594, 8762 and 3771) populations are classified as 3A, 4A and 4A group respectively (Table 2).

Table 2. Karyotypic characters of different *Aegilops* taxa and populations

Taxon (population)	2n	A_1	A_2	%TF	DRL	VRC	SC	K.F.
A. *columnaris (4134)*	4x=28	0.623	0.114	26.72	2.62	7.92	4A	9sm+5st
A. *columnaris (4135)*	4x=28	0.554	0.194	30.01	5.09	7.63	3B	2m+9sm+3st
A. *columnaris (16500(*	4x=28	0.604	0.115	27.51	2.72	7.10	3A	12sm+2st
A. *tauschii (13938)*	2x=14	0.274	0.114	42.07	4.69	8.56	1A	7m
A. *tauschii (16379)*	2x=14	0.281	0.116	41.03	4.86	7.69	1A	7m
A. *tauschii (16426)*	2x=14	0.302	0.114	40.39	4.52	9.52	2A	6m+1sm
A. *triuncialis (16418)*	4x=28	0.635	0.127	26.30	2.93	8.44	4A	9sm+5st
A. *triuncialis (16577)*	4x=28	0.611	0.111	26.98	2.43	7.68	3A	11sm+3st
A. *triuncialis (15368)*	4x=28	0.620	0.088	26.65	1.97	7.01	4A	11sm+3st
A. *umbellulata (15594)*	2x=14	0.556	0.103	30.43	4.06	7.54	3A	5sm+2st
A. *umbellulata (8762)*	2x=14	0.584	0.105	28.89	4.63	8.40	4A	7sm
A. *umbellulata (3771)*	2x=14	0.596	0.084	27.83	3.45	7.33	4A	6sm+1st

2n: Diploid chromosome numbers A_1: intrachromosome asymmetry index, A_2: interchromosome asymmetry index, TF%: total form percentage, DRL: difference of relative length, VRC: value of relative chromatin, symmetry classes (SC) of Stebbins and karyotype formula (K.F.).

Figure 1. Representative mitotic plates of *Aegilops* – (a) *Aegilops tauschii* (13938), 2n=2x=14, (b) *Aegilops tauschii* (16426), 2n=2x=14, (c) *Aegilops tauschii* (16379) 2n=2x=14, (d) *A. umbellulata* (15594), 2n=2x=14, (e) *A. umbellulata* (8762), 2n=2x=14, (f) *A. umbellulata* (3771), 2n=2x=14, (g) *A. columnaris* (16500), 2n-4x=28, (h*) A. columnaris* (4134), 2n=4x=28, (i) *A. columnaris* (4135), 2n=4x=28, (j) *A. triuncialis* (16577). 2n=4x=28, (k) *A. triuncialis* (16418), 2n=4x=28, (l) *A. triuncialis* (15368) 2n=4x=28.

According to the Stebbins bilateral table, the studied populations were symmetrically classified. In the species with 1A class, *A. tauschii* (13938 and 16379) possesses the lowest A_1 and the highest TF% values. In the species with the B classes all populations have the highest values of A_2 and DRL; therefore, all of them have more asymmetric karyotypes (Table 2).

The highest VRC amongst all populations was obtained for *A. tauschii* (16426) and the lowest was obtained for *A. triuncialis* (15368). Based on intrachromosomal asymmetry, some populations had the most asymmetrical and evolutionary karyotype (are classified as 4A). According to interchromosomal asymmetry, *A. columnaris* (4135) had the most asymmetrical karyotype in all of the populations. A statistical comparison based on completely randomized design demonstrates that there are significant differences among the populations for all the measured traits (P<1%) (Table 3). The principal component analysis (PCA), of the karyotypic traits shows that the first two components account for 88.18% of total variation. The first component (67.52 %) is positively correlated to the total chromosome length, short arm length, arm ratio, DRL, A_1 and TF% which had the highest coefficients of eigen vectors, while the second component (20.66%) accentuates

long arm length and A2 parameters (Table 5). Grouping of the populations are studied based on their relative karyotypic as well as mitotic characteristics (Table 4, Fig. 3). Cluster analysis based on the Average method was used to group the species and populations. The results showed that there was the minimum distance between two populations of *A. columnaris* (4134) and *A. triuncialis* (16577) and the populations classified under three groups which certainly the first and the second components had the most significant role in separated classes (Fig. 3).

The diagram of the populations' dispersion, based on two first components showed the populations separated in three groups, which completely fits with the results obtained through the average grouping analysis (Fig. 2).

Table 3. The results of variance analysis for karyotypic data based on CRD design

		Mean of squares								
S.O.V	D.F	TL	LA	SA	AR	CI	DRL	TF	A_1	A_2
Populations	11	2.71**	1.03**	2.23**	1.41**	0.02**	5.30**	191.85**	0.100**	0.003**
Error	48	0.62	0.30	0.07	0.03	0.0003	0.58	2.95	0.002	0.0003
%C.V.		9.99	10.31	10.48	7.66	5.48	5.83	5.48	7.97	8.39

**: Significant at 1%.

Table 4. Mean of chromosomes analysis of *Aegilops* populations

Populations	TL	LA	SA	AR	CI	DRL	%TF	A1	A2
A. columnaris (4134)	7.76 bc	5.64 ab	2.12 cd	2.67 ab	0.27 d	2.99 cd	26.72 d	0.61 ab	0.13bc
A. columnaris (4135)	7.46 bc	5.16 abc	2.30 cd	2.27 c	0.30 bc	5.38 a	29.97 bc	0.55 ab	0.20a
A. columnaris (16500)	6.91 bc	4.96 bc	1.95 cd	2.54 abc	0.28 bcd	2.91 cd	27.97bcd	0.59 ab	0.12bc
A. tauschii (13938)	8.56 ab	4.96 bc	3.60 a	1.38 d	0.42 a	4.73 ab	42.12 a	0.26 c	0.12bc
A. tauschii (16379)	7.57 bc	4.42 c	3.15 b	1.41 d	0.41 a	4.62 ab	41.40 a	0.27 c	0.11bc
A. tauschii (16426)	9.37 a	5.53 ab	3.84 a	1.44 d	0.40 a	4.78 ab	40.41 a	0.30d c	0.12bc
A. triuncialis (16418)	8.27 abc	6.05 a	2.22 cd	2.75 a	0.26 d	3.16 cd	26.19 d	0.62 a	0.14b
A. triuncialis (16577)	7.65 bc	5.56 ab	2.09 cd	2.66 ab	0.27cd	2.57 d	26.85 cd	0.60 ab	0.11bc
A. triuncialis (15368)	6.72 c	4.90 bc	1.82 d	2.69 ab	0.26 d	2.37 d	26.43 d	0.61 ab	0.10c
A. umbellulata (15594)	7.54 bc	5.24 abc	2.29 cd	2.29 c	0.30 b	4.05 abc	30.42 b	0.54 b	0.11bc
A. umbellulata (8762)	8.24 abc	5.81 ab	2.43 c	2.41 bc	0.29 bcd	4.91 ab	29.02 bcd	0.56 ab	0.12bc
A. umbellulata (3771)	7.14 bc	5.10 abc	2.04 cd	2.50 abc	0.28 bcd	3.71 bcd	28.05 bcd	0.58 ab	0.10c

TL: total length of chromosome, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, DRL: difference of relative length, TF%: total form percentage, A_1: intra-chromosome asymmetry index, A_2: inter-chromosome asymmetry index.

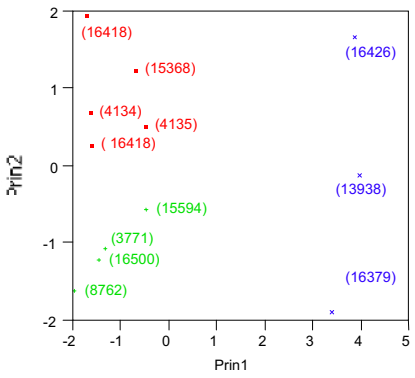

Figure 2. Scatter plot of 12 populations for the first two principals

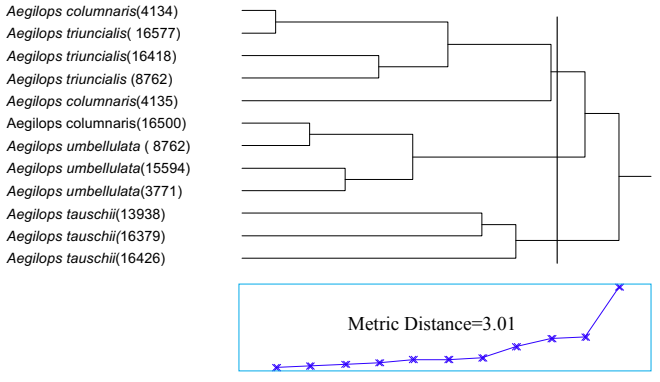

Figure 3. Dendrogram of 12 populations of *Aegilops* by analyzing nine karyotipic parameters using Ward 's cluster analysis method. Cophenetic correlation r=0.87

Table 5. Eigen vectors from the first two principal components for nine karyotype parameters to classify 12 populations of *Aegilops*

Parameters	First component	Second component
TL	0.25	**0.61**
LA	-0.17	**0.69**
SA	**0.41**	0.17
AR	**-0.43**	0.06
CI	**0.42**	-0.07
DRL	**0.34**	0.29
A1	**-0.43**	0.06
A2	-0.007	**0.32**
%TF	**0.42**	-0.07
Eigen Value	5.40	1.65
Percentage of Variance	67.52	20.66
Cum Percentage of variance	67.52	88.18

Discussion

The finding of this research reveals a detailed picture of the chromosome features in *Aegilops* species. Numerous reports, including those of (Bor 1968; Lange and Jochemsen 1992a, 1992b) have shown that the basis chromosome numbers for *Aegilops* genus no different basis chromosome among the different species (x=7) and ploidy levels are varied. In this study, twelve populations of four species of *Aegilops* possessed x=7 (2n=2x=14, 2n=4x=28).

Analysis of karyotype formulae showed that in *A. tauschii* species (2n=14) the number of "m" chromosomes is more than "sm" chromosomes, *A. umbellulata* species (2n=14) possessed the most "sm" type chromosomes. In tetraploid species, some species such as *A. columnaris* and *A. triuncialis* had the number of "sm" chromosomes more than "st" chromosomes.

At the interspecific level, quantitative and qualitative data allowed us the differentiation of several of the taxa studied. Among species, the most variable characters were the number of "m", "sm" and "st" chromosomes, as well as the number and position of satellites. (Fig. 1; Table 2). As a result, the species also could be differentiated by the number, type, and position of satellites.

Grouping of the populations based on taxonomic sections, confirmed the sections which introduced by Eig (1929) and Zhukovsky (1928).

The ratio of long arm /short arm chromosomes (AR) showed a high significant difference among some species belong to different sections, while other species are not clearly distinct (Table 4). Diploid species of *A. tauschii* (13938) for instance, had the lowest AR value (1.38), the highest TF% value (42.12) and the lowest A_1 value (0.26), exhibiting the most symmetrically karyotypes, while *A. triuncialis* (16418) with the highest AR value (2.75), the lowest TF% value (26.19) and the highest A1value (0.62) were introduced as the most asymmetrical karyotypes (Table 4). The pattern of variation of A_1 and A_2 values has been compared with the pattern of Stebbins' system in this study. In view of the fact that, fewer DRL value illustrated more symmetry of karyotype, *A. columnaris* (4135) and *A. triuncialis* (15368) respectively with DRL 5.38 and 2.37 values had the most symmetric and asymmetric karyotypes. Similarly, high DRL value leads to more changes in the construction of chromosomes.

The Duncan's test applied to the chromosome morphometric traits (LA, SA, TL, AR, DRL, TF%, A_1 and A_2) showed a highly significant difference among all examined populations belongs to different sections (Table 4).

The study revealed cytogenetic differences ($P<1$ %) in ANOVA for the karyological date as well as the ratio of long arms to short arms among diploid and tetraploid populations. So

these results indicate a significant quantitative change in the amount of chromatin in *Aegilops* species diversification (Table 2 and Table 3).

Considering the changes of interchromosomal asymmetry index (A_2) among diploid and tetraploid species, the lowest value exists in the diploid and some tetraploid species and the highest value only exists in the tetraploid species (*A. columnaris*) (Table 4).

Cluster analysis based on cytological data showed the populations with the lowest metric distance may lead us to use populations in crosses for inducing the highest genetic variations (Fig.3). However, the grouping of the *Aegilops* populations based on karyotypic data, partly agrees with either the taxonomic treatment of the genus *Aegilops* (Eig 1929 and Zhukovsky 1928) or phylogenetic analysis of the same species based on morphological characters.

Different populations of all species are classified as the same group except for *A. columnaris* (16500) majorly because of their different chromosome long arm and their DRL index. The present study shows the change in the chromosomal traits as one of the mechanism of inter and intraspecies diversification in the *Aegilops* genus as well as the earlier cytological reports. The differences in karyotype formulae and asymmetric indices found among the species suggest that structural changes of chromosomes may contribute to the diversification of the genus. These genomic differences could be used for breeding purposes. In general, cytological studies of the *Aegilops* species growing in Iran indicate the importance of polyploidy, chromosome structural changes, presumably quantitative changes in the amount of DNA and probably the role of growing sites in species diversification and suggest that such data may be used in the taxonomy and phylogenetic consideration of the genus.

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Morphological remarks on four species of the genus *Polygonum* L. from Istanbul (Turkey)

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Abstract

The genus *Polygonum* (Polygonaceae) contains almost 300 species in the world. It consists of 41 species as seven sections in Turkey. A number of *Polygonum* species are used as food and for traditional folk medicines**.** It is one of the difficult genera for taxonomical identification. In this study, morphological characteristics were examined on four species of the genus *Polygonum* from Istanbul (Turkey) and were presented with photographs of their leaves, ocreae, flowers, and seeds; *P. aviculare, P. patulum subsp. pulchellum, P. lapathifolium, and P. istanbulicum* (endemic). Also, collected specimens were compared with ISTE (The Herbarium of the Faculty of Pharmacy, Istanbul University) specimens.

Keywords: *Polygonum*, endemic, morphology, Istanbul, Turkey.
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İstanbul'dan (Türkiye) toplanan dört *Polygonum* L. türüne morfolojik katkılar

Özet

Polygonum cinsi dünya üzerinde yaklaşık 300 kadar tür içermektedir. Türkiye'de 7 seksiyon halinde 41 türle temsil edilmektedir. Çok sayıdaki *Polygonum* türünün geleneksel tıpta ve gıda olarak kullanımı mevcuttur. Taksonomik açıdan tanımlanması zor olan cinslerden biridir. Bu çalışmada İstanbul'dan toplanan dört türün morfolojik özellikleri incelenmiştir ve yaprak, okrea, çiçekleri ve tohumlara ait fotoğraflar sunulmuştur. Bu türler şunlardır; *P. aviculare, P. patulum* subsp. *pulchellum, P. lapathifolium,* and *P. istanbulicum* (endemik).Ayrıca toplanan örnekler ISTE (İstanbul Üniversitesi, Eczacılık Fakültesi Herbaryumu)'deki örneklerle karşılaştırılmıştır.

Anahtar kelimeler: *Polygonum*, endemik, morfoloji, İstanbul, Türkiye.

Introduction

The genus *Polygonum* is a member of Polygonaceae family that contains, ca. 300 species and distributed worldwide in temperate climates (The Plant List 2016). A number of *Polygonum* species are used as food (Koçyiğit and Özhatay 2009; Özüdoğru et al. 2011) and for traditional folk medicinessuch as cardiovascularprotection (Howesand Perry 2011), antiinflammation (Fabricant and Farnsworth 2001), neuroprotection (Firenzuoli and Gori 2007) and mitigation of biochemical processes involved in age-related neurodegenerative disorders such as Alzheimer's (Ma et al. 2005) and Parkinson's disease (Halliwell and Gutteridge 1990). Chemical constituents recognized in the *Polygonum* species are flavonoids, triterpenoids, anthraquinones (Chakraborty and Duary 2014), coumarins (Savithramma et al. 2014), phenylpropanoids (Gürdal and Kültür 2013; Tuttolomondo et al. 2014), lignans (Hsu et al. 2007), sesquiterpenoids (Yıldırım et al. 2003; Intisar et al. 2013), stilbenoids (Lee et al. 2014), and tannins (Halliwell and Gutteridge 1990). Amongst them, flavonoids are the most common components found in the genus *Polygonum* and have previously been used as chemotaxonomic markers of the genus, also playing an important role in the systematics of the family Polygonaceae (Alam et al. 2014). The genus *Polygonum* called asknotweed. It consists of 41 species under seven sections in Turkey (Coode and Cullen 1967; Keskin 2012).

According to literature, stalks, seeds, and roots are the most common parts of this genus of herbs used in traditional medicine. It is the chemical composition, and pharmacological capacity of this group which also includes melliferous flower makes this genus's position more important among the other floras (Hsu et al. 2006). Moreover, this genus is considered as one of the difficult genus for taxonomical identification, which makes the classification daunting. In this study, morphological remarks were presented on four species of the genus *Polygonum* from Istanbul.

Materials and methods

The aerial and underground parts of *P. aviculare, P. patulum* subsp. *pulchellum, P. lapathifolium,* and *P. istanbulicum* were collected from the Maltepe & Çatalca regions of Istanbul during September and October 2013. The collected fresh samples were first driedby pressing with a plant press. Thereafter, the dried species were placed in a freezer for at least 3 days at -18°C. This step is crucial, as all species must be free from any pests. Later, the specimens were morphologically identified to genus, species level and by family. After identification, every specimen got a label and ISTE number. All the species were kept in ISTE. Four collected plants from different regions in Istanbul were compared to almost 30 other species of the *Polygonaceae* family that exist in ISTE (Table 1). The process of screening, sorting and manipulating the sampleswas performed using Stereo microscope (Leica S8APO) and morphological software application (Suite Version 2.8.1). Primary analysis of *P.aviculare, P. patulum* subsp. *pulchellum, P. lapathifolium, and P. istanbulicum* were done by comparing of each taxon with already existing analysis archived in the herbarium. Since a reliable analysis depends on identification of several morphological characteristics, following are the characteristics that were defined for each plant in this study:

i. Size (width × height cm), body structure and overall shape of the plant.

ii. Internodes' features and ocreae structure and their length were determined.

iii. Size and shape of the leaves bracts were defined.

iv. Features of the buds along with flower structure, flower size, color, and size of the nut were also defined.

v. Towards the end of the analysis, information about flowering time and habitat were also collected.

Table 1. Morphological information about four *Polygonum* species used in this study and archived in ISTE.

Polygonum aviculare			
ISTE number	**Locality**	**Date**	**Determination**
68339	A2(E) Maçka	10.06.1995	A.Baytop
6560	A1(E) Edirne	19.05.1961	T.Baytop
7865	A2(E) Üniversite Bahçesi	15.09.1964	G.Ertem
92412	A1(E) Demirköy	26.07.2009	E.Akalin
30711	A2(A) Çınarcık	4.08.1974	G.Dökmeci
7793	A2(E) Belgrat Ormanı	29.10.1964	A.Baytop
31733	A1(E) Kırklareli	25.05.1975	K.Alpinar
Polygonum patulum			
2622	A2(A) Gemlik	14.09.1954	A.Baytop
43470	A1(E) Enez	13.08.1979	E.Tuzlaci
63734	A1(E) Terkirdağ	28.10.1991	E.Akalin
43518	A1(A)	24.08.1979	E.Tuzlaci
10382	A1(E) Edirne	4.09.1966	A.Baytop
34056	A1(E) Kocag yolu	5.09.1975	K.Alpinaz
34054	A1(E) Babaeski	5.09.1975	K.Alpinaz
18474	A2(E) Güzelceköy	11.10.1970	A.Baytop
10429	A1(E) Edirne	5.09.1966	A.Baytop
31130	A2(E) İstanbul	29.10.1974	G.Cakiser
18474	A2(E) Güzelceköy	11.10.1970	A.Baytop
3764	A2(A) İstanbul	28.08.1950	T.Baytop
3765	A2(A) İstanbul	13.10.1950	T.Baytop
5773	A2(A) Maltepe	24.09.1959	A.Baytop
Polygonum lapathifolium			
62397	A1(E) Kırklareli: Pınarhisar	1.08.1990	A.Baytop
64698	A1(E) Tekirdağ: Karahisarlı Köyü	23.07.1992	E.Akalin
80924	A1(E) Kırklareli: Hamidiye köyü	25.06.2002	Sukran Kultur
22425	A1(E) Edirne	16.06.1972	A.Baytop
18479	A2(E) Güzelceköy	11.09.1970	G.Ertem
3133a	A2(E) Kemerburgaz, Bahçeköy	3.09.1952	A.Baytop
11599	A2(E) Karamandere	28.07.1967	A.Baytop
1826	A2(A) Bursa, Orhangazi	19.09.1948	A.Husnu Demiriz
23076	A2(A) Şile	11.08.1972	H.Arpöksel
3783	A2(A) Aydos	17.08.1950	A.Baytop
3782	A2(A) Büyükbakal	26.08.1950	A.Baytop
3132	A2(A) Ömerli Deresi	24.08.1952	T.Baytop
3134	A2(A) Şile	24.08.1952	T.Baytop
92411	A1(E) Kırklareli, Demirköy	26.07.2009	E.Akalin
57213	A2(E) Küçükçekmece	18.08.1986	K.Engezem
18725	A2(A) Kuş Cenneti	16.09.1970	A.Baytop
82277	A2(E) Çatalca	15.08.2003	I.Genc
Polygonum istanbulicum			
83798	A2(A) Maltepe, Başıbüyük Mah.	05.11.2006	M.Keskin

Results

Polygonum aviculare L.

Results from morphological analysis of *P. aviculare* showed that the plant is an annual herbs and prostrate or procumbent. The stems were numerous with branched base and above prostrate with a size of 5 − 60 cm and slight sulcate. The internodes were between 15 − 25 mm. The size of ocreae membrane was about 1.5 −2 mm and was veined with upper hyaline glabrous and entire when young but becoming lacerate upon maturing and shorter than internodes. Leaves were sessile and elliptic of size 1.5-2 × 8 -10 mm and green in both postulate and undulate positions.

Inflorescences were generally branched, spicate and dense. There were flowers of almost 2 −3 located at each node and pedicels were 1 −2 mm. The color of flowers belonging to this specimen was pink, and buds were recognized as pink as well. The size of the flowers was between 1.5 − 2.0 mm. The nut was brown in color with was between 1.5 −2 mm in size (Fig. 1).

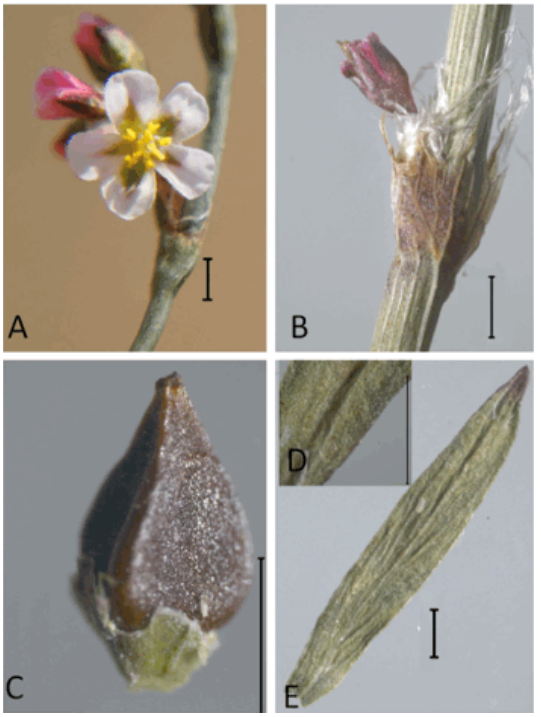

Figure 1. Morphology of ***Polygonum aviculare***: A) Flower, B) Ocreae, C) Nut, D) Surface of leaf, E) Leaf (Scale bar 1 mm).

Flowering time for this plant was estimated to be between July and November. The habitat belonging to *P. aviculare* was identified as barren at sea level of 700 m.

Polygonum patulum Beib. subsp. *pulchellum* (Lois.) Leblebici

Plan erect, flowering time is June to October. The size of the plant was between 20 − 50 cm. Stems were slender with tickness of less than 2.5 mm. The striate of the plant was white, and internodes were between 10-30 mm. The ocreae is shorter or longer than internodes and membrane of the ocreae was about 2 − 2.5 mm with a brownish color. The leaves were narrowly elliptic (10 − 45 × 3 − 8 mm) and longer than the bracts. Terminal inflorescences of the flowers were 1.5 − 2 mm in size. The nut of this plant was glossy and brownish, about 1− 1.8 ×1−1.5 mm (Fig. 2). The habitat belonging to *P. patulum* was recognized as open places at sea level of 1600 m.

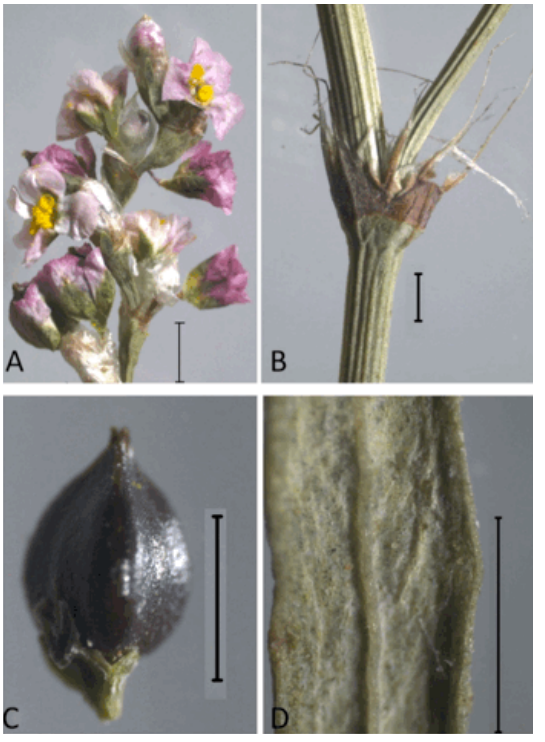

Figure 2. Morphology of ***Polygonum patulum***: A) Flower, B) Ocreae, C) Nut, D) Surface of leaf, E) Leaf (Scale bar 1 mm)

Polygonum lapathifolium L.

The morphological analysis of this plant confirmed that it is an annual herb with ascending stems and branches of size 10-50 cm and internodes of about 4 − 6 cm. The ocreae was shorter than internodes and membrane of the ocreae was about 4 − 6 mm with a hyaline glabrous. The leaves were narrowly elliptic with blackish spot and cuneate at the base. Peduncles were yellow and pink colored flower of size 1.5 − 2 mm. The nut of this plant was glossy, brownish, and about 1.8-2 × 1.5-1.8 mm in size (Fig. 3). The habitat belonging to *P. lapathifolium* was recognized as marshes at sea level of 1500 m. The flowering time between August and September.

Figure 3. Morphology of ***Polygonum lapathifolium****:* A) Flower, B) Ocreae, C) Nut, D) Surface of leaf, E) Leaf (Scale bar 1 mm).

Polygonum istanbulicum Keskin. (Endemic)

Results from morphological analysis of *P. istanbulicum* showed that this plant had a suffruticose perennial with a hard woody stock. The stems were branched at the base and were of the size of 5 −120 cm. The internodes were between 5 − 40 mm. The size of ocreae membrane was up to 10 mm and was veined with upper hyaline glabrous and entire when young but becoming lacerate upon maturing and shorter with a reddish brown color. Leaves were numerous with short but linear petiolate of size 5 −13 × 10 − 30 mm. The bracts were similar but smaller than cauline leaves. There were 2-3 flowers located at each node and had violet /pink color pedicels of 4 mm. Flowers were 2.5 − 4.0 mm in size and were of white color when young and became rose pink upon maturing. The nut was a brown color and 4× 5 mmin size (Fig. 4).

Figure 4. Morphology of ***Polygonum istanbulicum****:* A) Flower, B) Ocreae, C) Nut, D) Surface of leaf, E) Leaf (Scale bar 1 mm).

Flowering time for this plant was deduced to be between July to December. The habitat belonging to *P.istanbulicum* was recognized as open forest at sea level to 70 m.

Discussion

Since the genus *Polygonum* was revised by Coode and Cullen (1967) for the flora of Turkey, eight new species have been recorded (Davis et

al. 1988; Leblebici 1990; Tan and Baytop 1995), and five species new to science have been described from Turkey, (Leblebici 1985;Yıldız and Tan 1988; Yıldırımlı and Leblebici 1989, Zielinski 1991; Leblebici et al. 1993; Özhatay 2000). Eventually, *P. istanbulicum* described as a new to science (Keskin 2009).

They vary widely from prostrate herbaceous annual plants under 5 cm high, others erect herbaceous perennial plants growing to 3 − 4 m, and yet others perennial woody vines growing up to 20−30 m high in trees. Several are aquatic, growing as floating plants in ponds. The leaves are 1− 30 cm long, and vary in shape between species from narrow lanceolate to oval, broad triangular, heart-shaped, or arrowhead forms. The stems are often red-speckled or reddish. Flowers are small, white, pink or greenish, appearance in summer in dark cluster from the leaf joints or stem apices.The usual look of the leaves is mostly simple. However, stipules that forms on nodes and are known for their defensive role are mostly united around the stem. The spikes of flowers are found in varies forms like fascicles or panicles, hermaphrodite or unisexual and actinomorphic. In this genus, the wood and pollen are extremely different stated (Habibi et al. 2011; Mohammad et al. 2011).

Polygonum is a genus with indistinct taxonomic features between its members, systematic evidence obtained from a study of the features of the foliar epidermis, such as trichome morphology, is useful in providing significant distinctive characters for identification (Bunawan et al. 2011).

The results of our study revealed potentially important distinctive features of the four *Polygonum* species. These results suggest that more extensive study should be done on the micromorphology of the genus *Polygonum* to determine the usefulness of morphological features for making clearer taxonomic distinctions.

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Natural dye plants in Kepsut (Balıkesir, Turkey)

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Abstract

Natural dyes have traditionally been used in Anatolia for several years. An ethnobotanical study was conducted between 2012 and 2015 in order to determine wild plants used in Kepsut, which is a district of Balıkesir province in the Marmara region of Turkey. The purpose of this research was to get acquainted to know plants used as dyes in Kepsut, Balıkesir. The regional name of the plants, the parts used for dye production, and obtained colors have been reported. According to the results of the identification, 21 plant species belongs to 14 families were used as dye source in Kepsut. Used parts of species were aerial part, cortex, flower, fruit, gall, leaf, and root. Also 5 mixture receipts for dyeing were reported in Kepsut.

Key words: Ethnobotany, natural dye, Kepsut, Balıkesir, Turkey.
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Kepsut (Balıkesir, Türkiye)' da doğal boya elde edilen bitkiler

Özet

Doğal boyalar geleneksel olarak Anadolu'da yıllardır kullanılmaktadır. Kepsut'ta halk arasında kullanılan bitkileri belirlemek için 2012-2015 yılları arasında etnobotanik bir çalışma yapılmıştır. Kepsut, Marmara bölgesinde bulunan Balıkesir ilinin bir ilçesidir. Bu araştırmanın amacı, Kepsut ilçesinde halk arasında kullanılan doğal boya bitkilerinin tespit edilmesidir. Bitkilerin yöresel adı, boya eldesinde kullanılan bölümleri ve bitkilerden elde edilen renkler belirlenmiştir. Tespit edilen sonuçlara göre, Kepsut'ta 14 familyaya ait 21 bitki türü boya kaynağı olarak kullanılmaktadır. Türlerin kullanılan bölümleri toprak üstü kısımlar, kabuk, çiçek, meyve, mazı, yaprak ve kök kısımlarıdır. Ayrıca Kepsut ilçesinde boyama amaçlı kullanılan 5 farklı bitki karışımı da kaydedilmiştir.

Anahtar kelimeler: Etnobotanik, doğal boya, Kepsut, Balıkesir, Türkiye.

Introduction

Turkey has a rich potential from the natural dye plants. The dyeing of wool is conducted in three different ways: direct dyeing, dyeing in cubic way, and dyeing by the help of mediator substances. Wool fibers are dyed in one of those three ways. The dyes had been used until the end of the 18th century by local people. Wool fibers of carpets were dyed mostly with natural dye plants (Ozturk and Ozcelik 1991). Turkish people used different techniques of natural dyeing in the Middle Ages, and introduced natural dyeing to the world (Eyuboglu et al. 1983). The French people learned natural dyeing from the Turkish in 1715 (Atayolu, 1933). Kepsut is a district of Balıkesir Province in the Marmara region of Turkey. It is in the eastern part of Balıkesir (Fig. 1). It has an area of 894 km². The population is 24.180. Kepsut has 63 villages, some of which belong to Yoruks. Yoruks are especially very interested in natural dye plants. Karakeçili Yoruk communities live in 17 villages, Yağcıbedir Yoruk communities live in 9 villages (Fig. 2). In Yağcıbedir villages, carpet texture is a very old tradition. The Yağcıbedir Yoruks sell Yağcıbedir carpets to other cities of Turkey and also export to other countries (Fig. 5 and 6). Local people use natural dyes for dyeing the carpet yarn. In this study, traditional uses of dye plants in Kepsut (Balıkesir) were listed.

Figure 1. Map of Kepsut, Balıkesir, and Turkey.

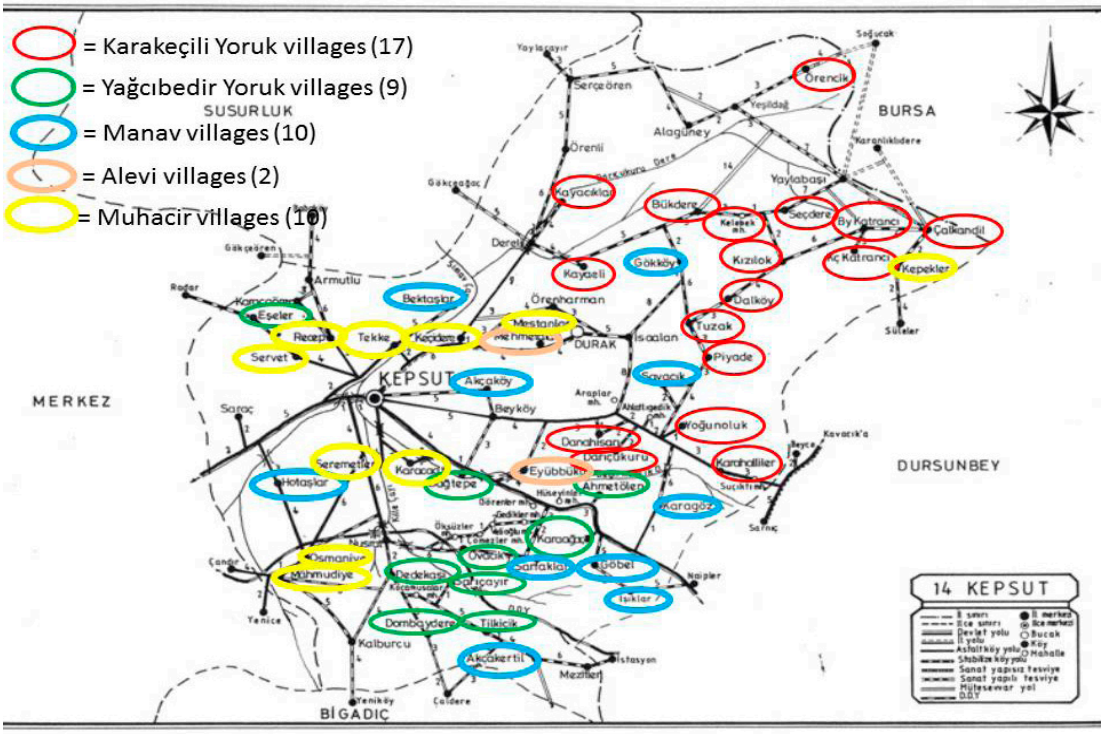

Figure 2. Ethnic characters of Kepsut villages.

Materials and methods

This research was performed between 2012 and 2015. The research area was in the Eastern part of Balıkesir. The 63 villages in Kepsut were visited during the research (Özdemir Nath 2016). Interviews were made with the local people (Fig. 3 and 4). A total of 305 individuals were interviewed in the area. Questions were asked about the natural dye plants. The local name of the plants, the parts used for dye production, and obtained colors have been reported. The uses of natural dyes in textile products such as cloth and carpet were reported. The dye plants were collected from nature with the help of the villagers. The collected plants were identified by using "Flora of Turkey and the East Aegean Islands" (Davis, 1965–1985; Davis et al.1988; Güner et al. 2000) and were cross-checked with the specimens kept at ISTE. The collected plant species were kept as herbarium reference at ISTE. Some plant species were deposited as a personal collection with the code of E.Ö.

Figure 3. Interview with the local people in Dedekaşı village, Kepsut (Balıkesir)

Figure 4. Interview with the local people in Karahaliller village, Kepsut (Balıkesir).

Figure 5. Yağcıbedir carpet of Kepsut (Balıkesir)

Figure 6. Yağcıbedir carpet small size, Kepsut

Results

This research allowed us to get information about dye plants in Kepsut, Balıkesir for the first time. There are 21 natural dye plants belonging to 14 families in Kepsut, Balıkesir. The local name of the plants, the parts used for dye production, and obtained colors have been listed (Table 1). The most commonly used parts of the dye plants were the cortex, aerial part, flower, fruit, gall, leaf and root. Also 5 mixture receipts for dyeing were reported in Kepsut (Table 2). Plant parts and water were put in boiler and were waited until desired colors were obtained. Some natural or chemical mediators are used for dyeing such as ash and salt.

Table 1. The natural dye plant species in Kepsut, Balıkesir.

Plant species, family and specimen number	Local names	Used part	Application	Color obtained
Achillea nobilis L. subsp. *neilreichii* (A.Kern.) Velen. (Compositae, ISTE 109654, 109624, 109655, 109656)	Ayvadana, Kurtotu	Areal part	Boiled with wool yarns of handmade carpet	Yellow
Alkanna tubulosa Boiss. Boraginaceae, ISTE 109578)	Kökboya	Root	Boiled with textile	Yellow, brown
Allium cepa L. (Amaryllidaceae, E.Ö. 4)	Soğan	Dried onion shells	Boiled with textile	Orange

Alnus glutinosa (L.) Gaertn. (Betulaceae, E.Ö.20)	Karaağaç, Kızılçınar	Cortex	Mixed with *Fraxinus ornus* and boiled with textile	Red
		Leaf	Used as a hand henna	Orange
Cistus laurifolius L. (Cistaceae, ISTE 109625, 109587)	Murt, Murtotu, Tavşanak, Tavşanaki, Tavşan pıynarı	Areal part	Boiled with onion shells and wool yarns of handmade carpet	Camel color
		Leaf	Boiled with wool yarns of handmade carpet	Blue, black, green
		Leaf	Boiled with wool yarns of handmade carpet	Keeps the color permanent on wool yarns
Fraxinus ornus L. (Oleaceae, E.Ö. 40)	Dişbudak	Cortex	Mixed with *Alnus glutinosa* and boiled with textile	Red
Helleborus orientalis Lam. (Ranunculaceae, E.Ö. 50)	Karacakökü, Karacaot, Kökboyası	Root	Boiled with wool yarns of handmade carpet	Red
Juglans regia L. (Juglandaceae, ISTE 109728)	Ceviz	Leaf	Boiled with cloth (Local name: Ferace,traditional village women cloth)	Black
		Bark of fruit, leaf	Boiled with wool yarns of handmade carpet	Black
		Bark of fruit, leaf	Mixed with henna for permanent color	Brown
Lavandula stoechas L. (Labiatae, ISTE 109852, 109826)	Karabaş otu, Kocabaşotu, Lavanta	Areal part	Boiled with wool yarns of handmade carpet	Bright pink
Malus sylvestris (L.) Mill. (Rosaceae, ISTE 109922)	Bayır elması	Bark of fruit	Boiled with textile	Pink
Primula vulgaris Huds. subsp. *rubra* (Sm.) Arcang. (Primulaceae, ISTE 109887)	Dağ marulu, Dere çiçeği, Karga basması, Karga yaşmağı, Marul	Root	Boiled with textile	Yellow
Prunus divaricata Ledeb. subsp. *divaricata* (Ledeb.) Schneider (Rosaceae, E.Ö.54)	Dağ eriği, Erik	Fruit	Fruit jam boiled with wool yarns of handmade carpet	Bright red

Quercus cerris L. (Fagaceae, ISTE 109685, 109687, 109690)	Ak gobak, Çalı gobağı, Gobak, Karakubak, Kara kombalak, Kızılmeşe, Kobak, Kobar çalısı, Kombalak, Kubak, Kubar, Meşe	Oak gall	Boiled with cloth (Local name: Ferace, traditional village women cloth)	Black
Quercus infectoria G. Olivier (Fagaceae, ISTE 109688, 109693, 109694, 109692)	Akgobak, Akmeşe, Akpıynar, Çalı kobağı, Gobak, Kasnak, Meşe, Pelit, Palamut, Sartal	Oak gall	Boiled with textile	Black
Quercus ithaburensis Decne. subsp. *macrolepis* (Kotschy) Hedge & Yalt. (Fagaceae, ISTE 109686, 109691)	Meşe, Kırmızı pelit	Cortex	Kept in water with textile and salt (traditional name Saçı kıbrıs) for 40 days, then washed with cold water	Claret red
		Cortex	Kept in water with goat leather used to put traditional cheese called tulum peyniri and salt (traditional name Saçı kıbrıs) for 40 days, then washed with cold water	Claret red
Rubia tinctorum L. (Rubiaceae, ISTE 109945, 109944)	Boyalık otu, Kökboya, Yapışkan ot	Root	Boiled with wool yarns of handmade carpet	Red
Rubus idaeus L. (Rosaceae, ISTE 109898, 109897, 109903,109925)	Karantı	Root	Boiled with wool yarns of handmade carpet	Green
Rumex crispus L. (Polygonaceae, ISTE 109883, 109884)	Alabardağı, Ebe kuzulağı, Eşek alabadası, Labada	Root, flower	Boiled with textile	Claret red
Salvia fruticosa Mill. (Labiatae, ISTE 109807, ISTE 109805, ISTE 109792)	Adaçayı, Boş, Boşotu, Boşapla, Muşapla, Moşapla, Puşapla, Şapla, Yakıotu	Aerial part	Boiled with textile	Red
Verbascum lasianthum Boiss. ex Benth. (Scrophulariaceae, ISTE 109954)	Eşek kulağı, Mayasıl otu, Sığır kuyruğu, Sığır sidiği	Flower	Boiled with textile	Green, orange, yellow

Table 2. The natural dye plant mixtures in Kepsut (Balıkesir).

Mixtures	Plant species	Application	Color obtained
Mixture 1	*Prunus domestica* fruits, *Juglans regia* leaves, *Rubia tinctorum* roots, *Malva sylvestris* roots	Boiled with wool yarns of handmade carpet	Bright red
Mixture 2	*Rubia tinctorum* roots, *Populus* sp. cortex, *Punica granatum* young stems, *Spartium junceum* stems, *Viscum album* leaves mixed.	Boiled with wool yarns of handmade carpet	Brown Note: *Viscum album* makes the color more permanent.
Mixture 3	*Rhus coriaria* fruits, *Lavandula stoechas flowers*, *Cistus salviifolius* flowers, *Cistus laurifolius* leaves	Boiled with wool yarns of handmade carpet	Blue, black, green
Mixture 4	*Jugans regia* root, *Salvia fruticosa* leaves, *Quercus* sp. galls, *Rubia tinctorum* root, *Rhus coriaria* fruits	Boiled with wool yarns of handmade carpet	Brown, black
Mixture 5	*Salvia fruticosa* leaves, *Quercus* sp. galls powder, *Punica granatum* young stems and cortex	Boiled with wool yarns of handmade carpet	Red

Discussion

Recently, the importance of natural dyes has been increasing in a fast manner because of toxic and allergic reactions associated with chemical dyes (Grover and Patni 2011). Carpet factories cannot export the carpets dyed in synthetic dyes belonging to Azo group. The woollen yarns of the carpets are required to be dyed with natural dyes, otherwise the companies and the countries will miss an important export bazaar. On the other hand many of dye plants have antimicrobial activity, antibacterial and antifungal activity (Gerson 1975; Wagner et al. 1989; Hussein et al. 1997). Natural dyes have minimum side effects, non-toxic features, less pollution, and beneficial effects. Because of that, natural dyes should be used more in different areas such as textile, food, toy and medicine industries. Turkey has rich natural plant diversity as a natural dye source. Therefore, more detailed researches are needed to detect the real potency and availability of natural dye plants.

This study helps to preserve valuable information about dye plants in Kepsut (Balıkesir) which may be lost in the future.

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