Pak. J. Bot., 45(5): 1735-1742, 2013.

THE CHANGE IN ECOLOGICAL, ANATOMICAL AND ANTIMICROBIOLOGICAL PROPERTIES OF THE MEDICINAL PLANT *TILIA RUBRA* DC. SUBSP. *CAUCASICA* (RUPR.) V. ENGLER ALONG AN ELEVATIONAL GRADIENT

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Abstract

In this study, ecological, anatomical and antimicrobial properties of *Tilia rubra* DC. subsp. *caucasica* (Rupr.) V. Engler which has several medicinal characters were investigated. There were significant differences both seasonal period and localities, in terms of N, P concentrations. There were no significant differences with respect to soil factors expect P_2O_5 concentration of the studied localities. The leaf length and width was decrease with altitudinal increases. Anatomically, cuticle, leaf thicknesses and the number of epidermal cells increase with in altitude. The results of the antibacterial and antifungal screening of extracts the flower and leaf of *T. rubra* subsp. *caucasica* from different district were investigated by diffusion disc plates on agar and the agar dilution method. It was observed that the extracts of flower and leaf have antimicrobial activity.

Introduction

The local communities of different regions of the world have centuries old knowledge (Deeb *et al*., 2013; Shinwari *et al*., 2013) and traditional uses of most of the plants occurring in their areas have been documented (Nadeem *et al*., 2013). This indigenous knowledge of plants has been transferred from generation to generation (Shinwari & Qaisar 2011). However, efforts to document data about the most commonly used genus *Tilia* were still missing.

The genus *Tilia* L. is a member of the family *Tiliaceae* and is represented by four species in Turkey: *Tilia cordata* Miller, *T. rubra* D.C., *T. platyphyllos* Scop. and *T. argentea* Desf., *Tilia rubra* DC. subsp. *caucasica* (Rupr.) V. Engler spread in the east of the Black Sea Region, especially (Davis, 1966). The different parts of species are consumed as medical. The flowers of *Tilia* species are used for treatment of several diseases, including microbial infections for emetic and strengthening effects, and for increasing urine and decreasing tension (Baytop, 1994; Toker, 1994). Also, Linden flowers are well known and widely used in folk medicine.

Concentrations of nutrients in mature leaves can indicate the nutritional status of a plant (Shinwari *et al*., 2013a). For this reason, foliar analysis is a classic tool for diagnosis nutrient use efficiencies (Tamm, 1964; Mayor & Roda, 1992). Different species found in a certain area takes their nourishment elements in different concentration and accumulates in various tissues, which is mainly the leaf tissue (Pastor & Bockheim, 1984; Hussain *et al*., 2011). Jayasekera (1993) stated that senescence is an important adjustment for macro nourishment in tall plants' adaptation process to the environmental factors and should be used in the optimum level for adaptation. Also Killingbeck (1984) says that nourishment elements are absorbed adequately by leaves and are accumulated in nourishment elements' storage organs in the course of abscission and senescence. Usually macro element concentration in leaves (particularly N) has a close relationship with photosynthesis (Reich *et al*., 1995; Hussain *et al*., 2010).

Several authors have emphasized the effect of altitudinal variations in the plant morphogenesis (Sulkinoja & Valanne, 1987; Ayobangira & Ntezurubanza, 1987; Yousuf *et al*., 2008). The fruits of some Tilia species growing in Turkey were investigated morphologically and anatomically by Toker *et al.,* (1997).Tanker & Toker (1984) studied from the morphologically and anatomically of inflorescences and leaves of Tilia species grown in Turkey. Possible variations in the morphology and anatomy of *Tilia rubra* subsp. *caucasica* in relation to altitudinal variations of this species and size of tissues of leaves have not been studied.

Natural products have served in the past as abundant source of compounds proven to be useful in the treatment of human disease (Gul *et al*., 2012). A novel source of natural plant products fielt collections of which have yield extracts with antineoplastic, antimicrobial, antifungal and antiviral activities (Alan *et al*., 1992; Sarwat *et al*., 2012). The alcoholic extracts of the Tilia flowers have antimicrobial properties, especially against some types of bacteria that may cause oral cavity infections (Suciu *et al*., 1988). The bract and flower infusion is also employed against ailments of the upper respiratory tract, due to the expectorant and antiseptic action of its constituents (Schulz *et al*., 2001).

In this study were investigate ecological, anatomical and antimicrobial properties of *T. rubra* subsp. *caucasica* in Turkey. The aim of this paper is to compare ecological, anatomical and antimicrobial properties which are different elevational gradients and different seasonal periods. Similar studies have been conducted by Walter *et al*., (2011) and Shinwari *et al*., (2013).

Materials and Methods

T. rubra subsp. *caucasica* specimens were collected from three natural populations along an elevational gradient from 20m to 1300m. of Ordu, which is situated on the north-eastern part of Turkey (A6 square based on the grid system of Davis). Mean annual temperature and precipitation is 14.1°C and 1013.5mm, respectively. Precipitation regime is A. W. Su. Sp., (A= autumn, W= winter, Sp= spring, Su= summer) and oceanic climate is seen in the study area.

All the plant specimens were collected from 0.25m x 0.25m. Quadrates located at random in each of the three populations. At least 30 plant specimens were taken from each site. Soil samples were also taken from the three populations and physical and chemical analysis (pH, total salinity, P_2O_5, K_2O, organic matter) was carried out according to standard methods (Kaçar, 1972).

Morphological features were identified from fresh and herbarium material. Observed results were compared with the Flora of Turkey (Davis, 1966). For ecologic analysis, plant samples were collected from three localities during vegetative, generative and senescence period. Soil samples were collected in generative phase. The parts of plant samples were dried at 70°C in an over for 48 h and milled. Nitrogen and phosphorus in plant samples were determined by standard methods (Allen *et al*., 1976). pH, salt, texture, phosphorus, potassium and organic matter were determined according to Öztürk *et al*., 1997. Ecological results have been examined by using one-way analysis of variance (ANOVA) test.

For anatomical analysis, cross-sections of leaves were used. Photographs of them were taken with a Nikon FDX-35 microscope. The stomata index and stomata index rate were calculated as described by Meidner & Mansfield (1968).

Preparation of extracts: Fresh leaves and flowers of the plants were dried at 45°C for 5-6 hours. The extract of the plants were prepared according to the methods described by Ertürk & Demirbağ, 2003 and Holopainen *et al*., 1988, with slight modification. Dried leaves and flowers of the plants were separately extracted with 95% ethanol (50g 1/5 ethanol) at room temperature. The extracts were kept at 4°C for a day, and they were filtered through 45 µm membrane filters, and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Test strains and culture media: Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of different 6 crude extracts of *Tilia rubra* subsp. caucasica L. were assayed against *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterobacter aerogenes* (ATCC13048), *Salmonella typhimarium* (ATCC5445), *Candida albicans* (ATTC 60192) and *Aspergillus niger*. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Brot (Merck). *C. albicans* and *A. niger* were grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentration of bacterial suspensions were adjusted to 10^8 cells/ml, and fungal suspension to 10^7cells/ml.

Antifungal and antibacterial assay: Antifungal and Antibacterial activity was measured using methods of diffusion disc plates on agar Ronald, 1990). In order to test Antifungal activity, the fractions of spices extracts were dissolved in 70%. Twenty milliliter of Sabouraud Dextrose Agar (Oxoid) was poured into each 15 cm Petri dish. *C. albicans, A. niger* were grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48. Growth was adjusted to OD (600 nm.) of 0.1 by dilution with Sabouraud Dextrose Broth (Difco). One hundred micro liter of suspension with approximately 108 bacteria per milliliter was placed in Petri dishes, over agar and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 15µl of each spices samples (1 g/ml). One hundred units of nystain were used as a positive control and alcohol as a negative control. For bacteria, as positive control Amiktodalin and Cefozin 10µl (40 mg/mL) and as negative control 70% alcohol were used. Antifungal assay was made the same method but only instead of Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid) used to Mueller Hinton Broth medium (Merck) and Mueller Hinton Agar medium (Merck).

Inhibition zones were determined after incubation at 27-37°C for 24- 48 h. All tests were made in triplicate.

Minimal inhibition concentration (MIC) for assay: The agar dilution method, describe by Vanden Berghe & Vietinck (1991) was used for the antibacterial screening with slight modifications. Instead of 96 well microtittre plates 24 well tissue culture (Corning) plates were used. The crude extracts were dissolved in 70% ethanol and physiological Tris buffer (Amresco 0826-500G) 1:4 and mixed with an equal amount of 3% agar solution at 45°C to a final 40, 20, 10, 5, 2,5 and1 .25 mg of extracts/ml from the solutions 400 µl was transferred into each well of the tissue culture plate. After solidification each well was inoculated with 10µl of freshly prepared bacterial suspension of 10^8 bacterial/ml and incubated at 37°C for 24 h. Antifungal assay was made the same method but only instead of Mueller Hinton Broth medium (Merck) and Mueller Hinton Agar medium (Merck) used to Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid) The bacterial and fungi growth was assessed by a stereo microscope after the incubation period. All tests were made in triplicate.

Results

Ecological properties

N- P Concentration: The % N and % P values are given Tables 1 and 2. The min- max nitrogen concentration of *T. rubra* subsp. *caucasica* was (3,14±0.25)-(3, 47±0.24) in the vegetative period, (2,31±0.24)-(3,47±0.37) in the generative period and (2,32±0.57)-(3,63±0.21) in the senescence period. The % P values were (0,047±0,021)-(0,063±0,028) in the vegetative period, (0,042±0, 0034)-(0,052±0,009) generative period and (0,044±0,022)-(0, 06±0,029) in the senescence period (Tables 1, 2).

Soil: The soil properties of *T. rubra* subsp. *caucasica* are given Table 3. This species generally prefers clayey-loamy and clayey soils. According to the results presented in Table 3, pH values were 7.18-7.96(7.48±0.242) so plant prefers slight alkali soils. Organic matter, P_2O_5, K_2O and total salt values were 2.02-5.02(3.73±0.89), 10.179-55.647(26.75±14.5), 234. 600-238 (236.33±1.20), 0.03-0.08(0.44±0.014), respectively.

Table 1. Concentration of N in different periods of plant samples.

N % localities	*		**		***	
	Min.	Max.	Min.	Max.	Min.	Max.
Locality 1	2,8	3,01	2,44	3,06	1,19	3,8
Locality 2	2,99	3,57	2,09	3,14	2,87	3,21
Locality.3	3,62	3,83	2,4	4,21	2,89	3,88
Mean ± SE	3,14±0,25	3,47±0,24	2,31±0,11	3,47±0,37	2,32±0,57	3,63±0,21

*Vegetative period, **Generative period, *** Senescence period

Table 2. Concentration of P in different periods of plant samples.

P % localities	*		**		***	
	Min.	Max.	Min.	Max.	Min.	Max.
Locality 1	0,005	0,006	0,003	0,0089	0,0009	0,0041
Locality 2	0,069	0,093	0,059	0,064	0,067	0,097
Locality.3	0,067	0,091	0,065	0,093	0,063	0,08
Mean ± SE	0,047±0,021	0,063±0,028	0,042±0,034	0,052±0,009	0,044±0,022	0,06±0,029

*Vegetative period, **Generative period, *** Senescence period

Table 3. Analysis results of the soil samples.

Localities	% Water saturation	% Total salt	pH	P_2O_5 (ppm)	K_2O	% Organic Matter
Locality 1	45 Clayey	0.03 Saltless	7.18 Slight alkali	10.179 High	234 High	4.16 High
Locality 2	63 Clayey-Loamy	0.08 Saltless	7.96 Slight alkali	14.427 High	237 High	2.02 High
Locality 3	67 Clayey-Loamy	0.05 Saltless	7.30 Slight alkali	55.647 Very high	238 High	5.02 High
Mean ± SE	58.33±6.77	0.44±0.014	7.48±0.242	26.75±14.5	236.33±1.20	3.73±0.89

Anatomical properties

Anatomy of Leaf: There is a single layered epidermis on the upper and lower surface of the leaf. Upper epidermis cells are larger than lower ones. There are mucilage cells on the upper surface. Epidermis cells have druses and monohydric crystals. Leaf is bifacial. Palisade cells are single layered and rich in chloroplasts. Spongy parenchyma cells are 2-3 layered. Vascular bundles are collateral type. Stomata type is anomocytic. Stomata are only present on the lower epidermis. The number of stomata is 52.8±2 and the number of epidermis cells is 142.8 on the lower epidermis of the leaf in Locality 1. The stomata index is 26.9. In Locality 2, the number of stomata is 48±2, the number of epidermis cells is 156 and the stomata index is 23.5 on the lower epidermis of the leaf. In Locality 3, the number of stomata is 45±2, the number of epidermis cells is 180 and the stomata index is 20 on the lower epidermis of the leaf. The thicknesses of leaf and some leaf tissues and stomata features at different altitudes are given in Tables 4 and 5 (Figs. 1-3). The width and length of leaves from different altitudes are given in Table 6.

Table 4. Morphological measurements of leaves of *T. rubra* subsp. *caucasica.*

Locality	Altitude (m)	Number of measured plants	Mean width of leaves (cm)	Mean length of leaves (cm)
1	20	30	8.454 ± 0.510	11.520 ± 0.722
2	600	30	7.98 ± 0,337	10.736 ± 0.472
3	1300	30	6.246 ± 0.329	8.66 ± 0.402

Table 5. Cuticle and leaf thickness of *T. rubra* subsp. *caucasica*.

Locality	Altitude (m)	Number of measured plants	Mean cuticle thickness(µ)	Mean mesophyll thickness(µ)	Mean leaf thickness(µ)
1	20	30	3.8 ± 0.560	93 ± 6.81	127.5 ± 6.81
2	600	30	4 ± 0.612	97 ± 3. 39	131 ± 5.94
3	1300	30	5 ± 0.790	99 ± 4.07	134.5 ± 5.02

Table 6. Stomata features on the lower epidermis from different altitudes of *T. rubra* subsp. *caucasica*.

Locality	Altitude (m)	Mean number of epidermal cells	Mean number of stomata	Stomatal index	Mean stomata width (µ)	Mean stomata length (µ)
1	20	142.8 ± 3	52.8 ± 2	26.9	16 ± 0.42	27 ± 1.45
2	600	156 ± 3.5	48 ± 2	23.5	14.5 ± 0.23	24 ± 3.34
3	1300	180 ± 4	45 ± 2	20	15 ± 1.68	29 ± 0.64

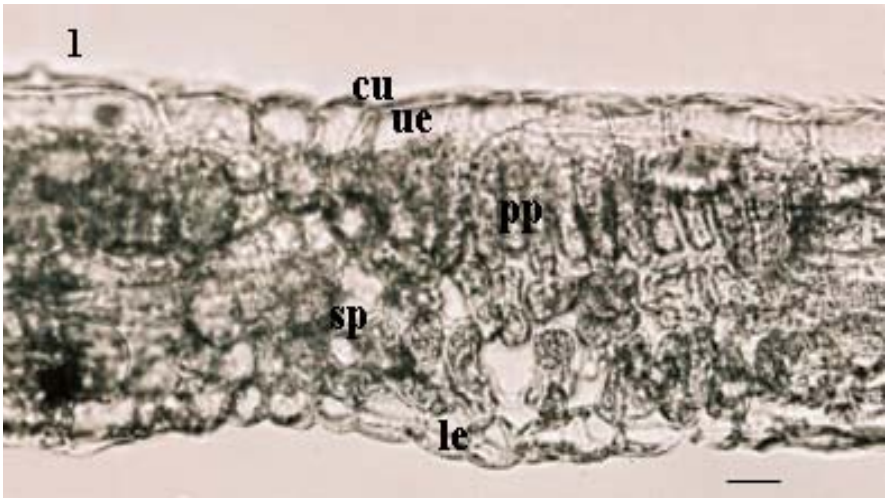

Fig. 1. *T. rubra* subsp. *caucasica* Cross-section of leaf (20 m). cu) cuticle, ue) upper epidermis, pp) palisade parenchyma, sp) spongy parenchyma, le) lower epidermis (Bar: 50 µ).

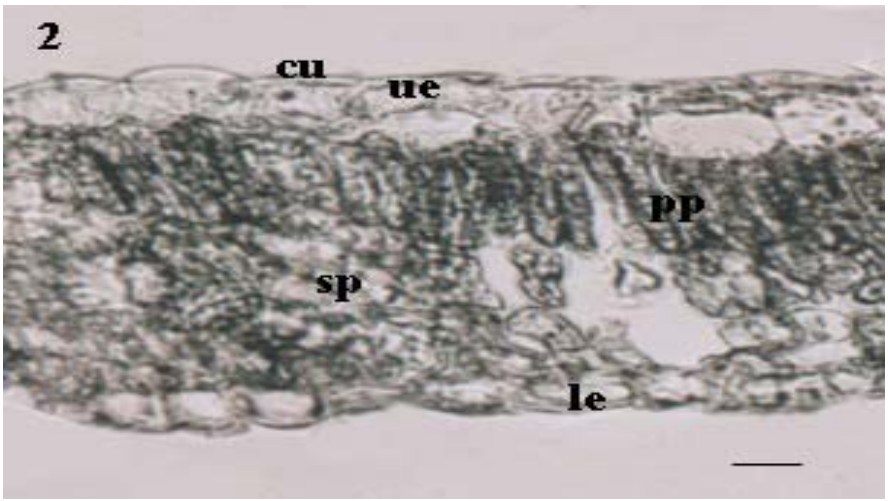

Fig. 2. *T. rubra* subsp. *caucasica* Cross-section of leaf (600 m). cu) cuticle, ue) upper epidermis, pp) palisade parenchyma, sp) spongy parenchyma, le) lower epidermis (Bar: 50 µ).

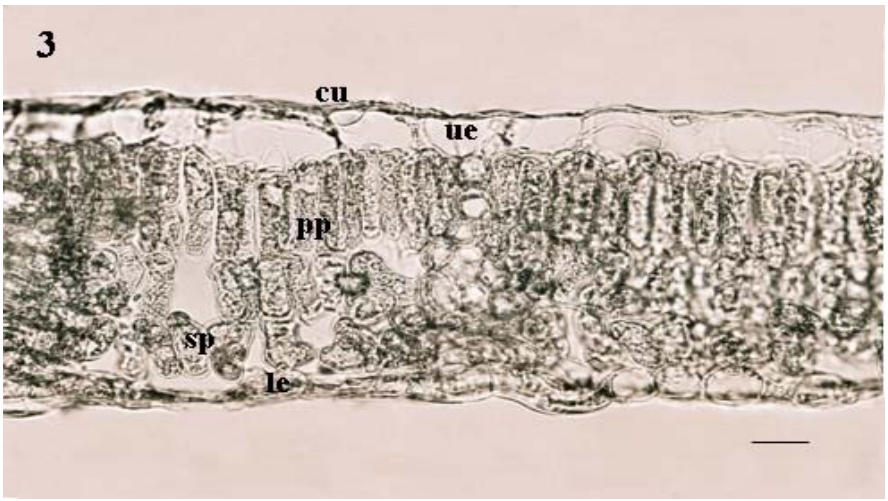

Fig. 3. *T. rubra* subsp. *caucasica* Cross-section of leaf (1300 m). cu) cuticle, ue) upper epidermis, pp) palisade parenchyma, sp) spongy p.

Antimicrobial properties: The results of the antibacterial and antifungal screening of extracts the flower and leaf of *T. rubra* subsp. *caucasica* from different district are reported in Table 7. Antibacterial activity of extract obtained from Linden flowers and leaf (gather from Locality 1) weakly showed antibacterial activity (7-15 mm/15 µl inhibition zone) against test organisms. But highest show antifungal activity against *A. niger* and C.*albicans* (15-16 mm/15 µl inhibition zone). However antimicrobial activity of extract obtained from Linden flowers and leaf (gather from Locality 2) showed antibacterial and antifungal activity (8-18 mm/15 µl inhibition zone) against test organisms. Antibacterial activity of extract obtained from Linden flowers and leaf (gather from Locality 3) highest showed antibacterial and antifungal activity (11mm/20 µl inhibition zone) against test organisms (Table 7).

The crude extracts of *T. rubra* subsp. caucasica (gather from Locality 3) required a MIC for against test organisms. of % 2.5-20 mg/mL concentration. Antibacterial activities of the crude extracts of *T. rubra* subsp. *caucasica* (gather from Locality 1and Locality 2) were similar, whereas the activities against the fungus differed slightly (Table 7).

The crude extracts of *T. rubra* subsp. caucasica from Linden' flower are more activities than leaf. In generally the crude extracts of *T. rubra* subsp. *caucasica* (gather from Locality 3) are more effective against bacteria and fungi than the other.

Discussion

Many environmental factors may affect plant development. Also the elevation gradient is an environmental factor (Mahmood *et al*., 2013). The amounts of precipitation, temperature, humidity and radiation change according to altitude. All these changes affect ecological, anatomical properties and contents of plants (Koçman, 1989; Tabanca *et al*., 2004; Kuroyanagi *et al*., 2012).

According to ANOVA test, no significant changes are determined in leaf nitrogen concentration along the elevational gradient. However, significant changes are determined statistically along the elevational gradient in terms of phosphorus ($p<0.01$). The lack of statistically significant differences with respect to nitrogen concentration is the dispersant effect of the carbon in leaves or luxury consumption (De Mars & Boerner, 1997).

There are statistically important differences in the three populations with respect to pH, total salinity, P_2O_5, K_2O, organic matter ($p<0.05$).

Nourishment elements in ripe leaves are the indicators of a plant's nutrient condition. For that reason, analyzing of leaves is a classical method for diagnosis of the nutrient production and mostly applied to forest trees. One of the most important methods used for measuring the effective consumption of nutrients in a plant is determining the nutrient absorption from the leaves to the storing organs throughout the growing up period (Killingbeck, 1988). Being moderate to this hypothesis a few studies explain the leaves' nutrient consumption for phosphate and nitrogen (De Mars & Boerner, 1997).

Whittaker (1956) has proved the topographic condition and humidity diversity and nutrient conditions' change in a forest. Along the elevational gradient, the samples of nourishment movements throughout the inner parts explain the usable land productivity, plant nutrient content and absorption relation hypothesis.

It has been determined that the samples taken from locality 3 commonly have a higher concentration in point of all macro elements in comparison to the other regions. It is claimed that the species in upper parts have higher nourishment element concentrations in comparison to the species of lower parts in a forest system that loses leaves (Tanner, 1985).

In the collected samples, significant differences are determined in point of growing season. Photosynthetic capacity decreases in leaves in the growing old period after the ripening period (Feller & Fisher, 1994) and especially macro elements like N, P are moved to leaves' ligneous parts. N nourishment element concentration measured throughout the growing up season demonstrates a decrease at the end of senescence.

In this study *Tilia rubra* species are commonly found in the argillaceous-loamy and loamy soils. PH percentages are slightly alkali. Salinity quantity is unsalted or lightly salted and no toxic effect on plants is under consideration. The quantity of phosphorus, organic substance and potassium is high.

The fruits, inflorescences and leaves of some *Tilia* species growing in Turkey were investigated morphologically and anatomically by Tanker & Toker (1984) and Toker *et al*., (1997). But they have not given any data about size of tissues of leaves. Possible variations in the morphology and anatomy of *Tilia rubra* subsp. *caucasica* in relation to altitudinal variations of this species have not been studied. Our results generally agree with Tanker & Toker (1984). The size of tissues of leaves and their altitudinal variations are reported here for the first time.

Many environmental factors change due to changes in altitude. For instance, the amounts of precipitation and radiation increase with an increase in altitude together with the effects of wind, daily temperature differences, cloudiness and humidity. On the other hand, evaporation and mean temperature decrease with an altitudinal increase and vegetative phases and the process of pedogenesis shorten (Koçman, 1989). All these changes in relation to altitude, affect plant life, in particular the morphological and anatomical characteristics (Gönüz & Özörgücü, 1999).

Morphologically, a decrease in the leaf length and width was observed in parallel with altitudinal increases. Noitsakis *et al.,* (1990) have reported that the size is reduced in Kermes oak under drought conditions. Anatomically, both cuticle and leaf thicknesses increase with in altitude. Also the number of epidermal cells increases with altitude on both upper and lower surfaces of the leaf. It was obtained that the number of stomata on the lower epidermis is decrease in high attitude. The stomatal index is 26.9 for Locality 1, 23.5 for Locality 2 and 20 for Locality 3. Gönüz & Özörgücü (1999) have studied possible variations in the morphology, anatomy, ecology and penology of *Origanum onites* L. in relation to altitudinal variations. They have reported that the thicknesses of the cuticle and the whole leaf increase with an increase in altitude. Also they have stated that in general the number of stomata and epidermal cells changes with altitude. Our results are similar their results.

Table 7. Results of antimicrobial screening of spice plant extracts determined by the agar-well diffusion method (minimum inhibitory concentration, MIC, in mg/ml) and agar diffusion method (inhibition zone in mm).

Plant species and family	Part used	Collection site	Altitude (m)	Collection time	Microorganisms															
					Inh. Zone (mm)								MIC (mg/ml)							
					E.c	S.a	S.e	S.t	E.a	P.a	C.a	A.n	E.c	S.a	S.e	S.t	E.a	P.a	C.a	A.n
Tilia rubra subsp.*caucasica* (*Tiliaceae*)	Leaf	Locality 1	(20m)	July (2003)	7	12	7	15	10	8	15	16	2	2	4	2	4	4	2	1
	Flower	Locality 1	(20m)	July (2003)	8	10	8	11	8	7	14	18	4	4	2	4	8	8	8	0.5
	Leaf	Locality 2	600 m	July (2003)	10	15	10	13	12	11	16	15	4	1	2	2	2	4	0.5	1
	Flower	Locality 2	600 m	July (2003)	8	12	8	9	10	12	18	14	4	2	2	2	4	2	1	2
	Leaf	Locality 3	1300 m	July (2003)	15	12	13	14	16	13	14	18	1	0.5	0.5	1	4	4	0.5	0.5
	Flower	Locality 3	1300 m	July (2003)	12	20	11	12	15	11	10	15	2	0.5	1	1	2	2	1	1
	Amiktodalin				25	30	25	20	25	22	ND	ND	NT	NT	NT	NT	NT	NT	NT	NT
	Cefozin				20	36	22	20	25	20	ND	ND	NT	NT	NT	NT	NT	NT	NT	NT
	Nystatin				ND	ND	ND	ND	ND	ND	16	15	NT	NT	NT	NT	NT	NT	NT	NT
	% 70 Alkol				–	–	–	–	–	–	–	–								

-: No inhibition, ND: NT: Not tested,
Part used: Fr, flower; Lf, leaf;
Microorganisms: E. c., *E. coli;* P.a., *P. aeruginosa;* B. s., *B. subtilis;* S. a., *S. aureus, C. albicans, A. Niger*

The variations in plant structure that are commonly affected by environmental factors are particularly strongly expressed in the morphology and anatomy of leaves. The leaf has often been considered the most anatomically variable organ of the plant and leaf adaptations have been used as indicators of environmental conditions (Dickison, 2000).

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purposes due to decrease in natural richness and drawbacks. Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various diseases in Turkey.

In this study, the antimicrobial capacity of the extracts from the flower and leaf of *T. rubra* subsp. *caucasica* against bacteria and fungi were determined. The antimicrobial activity of the extracts of flower and leaf was more effective against bacteria than fungi, similar to the results of Avato *et al*., (1997) and Zavala *et al*., (1997). But the antimicrobial activity of the extracts of bark from *Tilia* species was more effective against fungi than bacteria Toker *et al*., (1995).

The use of some antibiotics is no longer recommended because of the potency of the widespread resistance to them (Zavala *et al*., 1997). Thus, flower and leaf of *T. rubra* subsp. *caucasica*, like many other plants, can be used in place of antibiotics. Thus, flower and leaf of *T. rubra* subsp. *caucasica*, like many other plants, can be used in place of antibiotics. Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc., (Mehjabeen *et al.,* 2011). Especially the crude extracts of *T. rubra* subsp. *caucasica* from Linden' flower are more activities than leaf. In generally he crude extracts of *T. rubra* subsp. *caucasica* (gather from Locality 3) are more effective against bacteria and fungi than the other. The concentration and ratio of the active compounds in essential oils depend on the plant variety, origin, time of harvest, and conditions of processing and storage (Deans & Ritchie, 1987). The *Origanum majorana* L. (Lamiaceae) grown in the southwestern part of the Mediterranean region, was characterized by rich oil yield with high carvacrol content, but *O. majorana* grown in the western part of Turkey was poor in oil and contained only trace amounts of carvaerol (Tabanca *et al*., 2004).

The results of study show that the ecological, anatomical properties and microbial contents of some plants change according to elevational gradient and climatic condition (Escalona, 1991; Avissar, 1993; Tabanca, 2004). In our studies we noticed that both concentration of N, P nutrients, soil properties, cuticle and leaf thicknesses, number of stoma and epidermis cells and microbial contents change with increases in altitude.

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(Received for publication 9 March 2011)