

## **STUDIES ON THE ANATOMICAL, PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF ENDEMIC *RHODODENDRON SMIRNOVII* TRAUTV.**

ENDEMİK *RHODODENDRON SMIRNOVII* TRAUTV. 'NİN ANATOMİK,  
FİTOKİMYASAL VE ANTİMİKROBİYAL ÖZELLİKLERİ ÜZERİNDE ÇALIŞMALAR

**Nalan TEZGÜL ÇAKIR<sup>1</sup>, Ayşegül GÜVENÇ<sup>2</sup>, Gamze KÖKDİL<sup>3</sup>, Nizam DURAN<sup>4</sup> and  
Gönül ARSLAN<sup>5</sup>**

<sup>1</sup> Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandoğan-  
Ankara, TURKEY

<sup>2</sup> Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100,  
Tandoğan-Ankara, TURKEY

<sup>3</sup> Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenişehir Campus,  
33169 Mersin, TURKEY

<sup>4</sup> Mustafa Kemal University, Faculty of Medicine, Department of Microbiology, Antakya,  
TURKEY

<sup>5</sup> Mersin University, Faculty of Medicine, Department of Microbiology, Mersin, TURKEY

### **ABSTRACT**

*No previous detailed botanical study exist on Rhododendron smirnovii Trautv., which is endemic in Turkey. The aim of this study was to provide information on the anatomical properties of the leaf and stem of R.smirnovii. Anatomical studies include cross sections of the leaf and stem with illustrations. In addition, phytochemical screening methods were applied for identifying the major chemical groups in this species. The antibacterial activities of the leaf and stem extract of R.smirnovii were also investigated for three Gram (+) (Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis) and two Gram (-) (Escherichia coli, Pseudomonas aeruginosa) bacteria by employing both microdilution and disc diffusion method. Subsequently, inhibitory activity against yeast-like fungi (Candida albicans, Candida krusei, Candida glabrata and Candida parapsilosis) was also determined.*

*Anatomical features of the plant were described. Flavonoids, tannins and reducing sugars were detected in the leaf and stem of the plant. It was found that methanol extract of the leaf inhibited growth of the microorganisms at different level. The leaf extract posses more antibacterial activity than antifungal*

activity. The stem methanol extract of *R. smirnovii* did not show any antimicrobial activity against the bacteria and the fungi investigated in this study.

**Keywords:** *Rhododendron smirnovii* Trautv., leaf and stem anatomy, major chemical compounds, antimicrobial activity.

## ÖZET

Endemik bir tür olan *Rhododendron smirnovii* Trautv üzerinde yapılmış herhangi bir detaylı botanik araştırma bulunmamaktadır. Bu çalışmanın amacı bitkinin dal ve yapraklarının anatomik özelliklerini belirlemektir. Yaprak ve dallardan alınan enine kesitlerin çizimleri yapılmış ve fotoğrafları çekilmiştir. Buna ilaveten türün içerdiği major kimyasal gruplar da fitokimyasal tarama metotları kullanılarak saptanmıştır. *Rhododendron smirnovii*'nin dal ve yaprak ekstratlarının üç Gram (+) (*Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) ve iki Gram (-) (*Escherichia coli*, *Pseudomonas aeruginosa*) bakteriye karşı antibakteriyel etkileri ise disk diffüzyon ve mikrodilüsyon yöntemleri kullanılarak araştırılmıştır.

Ayrıca maya mantarlarına (*Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida parapsilosis*) karşı da etkileri test edilmiştir.

Anatomik özellikler tanımlanmış, dal ve yaprak ekstratlarında flavonoid, tanen ve redüktör öz varlığı saptanmıştır. Yaprığın metanollü ekstresi kullanılan bakterileri farklı düzeylerde inhibe etmiştir. Yaprak ekstresi antifungal etkiden daha kuvvetli antibakteriyel etkiye sahiptir. Dalın metanollü ekstresi herhangi bir antibakteriyel ve antifungal etki göstermemiştir.

**Anahtar Kelime:** *Rhododendron smirnovii* Trautv., yaprak ve dal anatomisi, major kimyasal bileşenler, antimikrobiyal aktivite

## INTRODUCTION

The genus *Rhododendron* belongs to Ericaceae family and is one of the largest, most widespread woody plant genera of dicotyledons. There are about 1025 species in the world. *Rhododendron* species are distributed from the northern temperate zone, throughout tropical South-east Asia, to North-eastern Australia (1, 2). The largest centres of the genus (up to 700 species) are in China, Tibet, Burma, Assam and Nepal. The genus also has a secondary center of almost 300 species in New Guinea. The rest of the species are found in the Himalayas and Japan, with a small number occurring in Europe, Southern Asia and North America. Many of the species are ornamental shrubs and are widely cultivated in gardens (1-3).

*Rhododendron* has been the subject of extensive taxonomic, phylogenetic and ecologic studies in recent years (4-7). The leaves, flowers, pollen and nectar of many *Rhododendron* species

contain toxic diterpenoid, and toxic honey produced by bees which have visited these flowers. The toxic honey is used by some herbalists as ingredient of medicine (8, 9). Besides toxic diterpenes, *Rhododendron* species contain also flavonoids, simple phenols and phenolic acids, triterpenoids, tannins and essential oils (10-17).

In Turkey, *Rhododendron* is represented 5 species and 12 taxa, four of which are hybrids (2, 18,19). These taxa become more common on the further eastward in forest glades in the Black Sea mountains. The first flowers appear in early May and continue until the end of June, or until mid-July at higher altitudes. There are a few study on *Rhododendron* species growing wild in Turkey in the literature. The ecological wood anatomy of Turkish *Rhododendron* L. have been previously reported (2, 7). A recent study of the volatile components of five Turkish *Rhododendron* species show that these species have an intraspecific variations in volatile compositions (20). Two pharmacological studies of *Rhododendron* species growing in Turkey have been done. The anti-inflammatory and antinociceptive activity of the leaves of *R. ponticum* was investigated (21). The acetylcholinesterase and butyrylcholinesterase inhibitory activity of the extracts of *R. ponticum* subsp. *ponticum* and *R. luteum* was also investigated by Orhan et al. 2004 (22). Antioxidant, antimicrobial and acetylcholinesterase inhibitory activities of *Rhododendron* species were studied by Tasdemir et al. using TLC bioautographic methods (23).

*R. smirnovii* Trautv. is an endemic species growing wild in the Eastern side of the black-sea (18). Anatomical structure of the leaf and stem of *R. smirnovii*, its major groups of chemical compounds and antimicrobial activity of the extracts have not been studied in detail. Therefore, the purpose of this paper is to investigate the anatomical features of the leaf and stem of *R. smirnovii*, its major groups of chemical compounds and antimicrobial activity of the methanol extracts of leaf and stem of the plant.

## **MATERIAL AND METHODS**

### **Plant material**

*R. smirnovii* Trautv. was collected during its flowering period in June 2000 from Artvin, Murgul-Gölbaşı plateau at the altitude of 2150-2200 m. Voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, University of Ankara, Turkey (AEF 212466).

### **Anatomical Studies**

The material used were stem and leaf preserved in 70% ethanol. Cross sections of stem and leaf were prepared by hand from preserved material in chloral hydrate and Sartur reagent. Sartur reagent contains KI-I, aniline, sudan III, lactic acid, alcohol and water (24). Illustrations were made

using a Leitz drawing prism attached to a Leitz-Wetzlar (45°) microscope. Photographs from the preparations were taken with a camera adapted to Olympus BX 50 microscope.

### **Phytochemical screening**

The extracts of leaf and stem of *Rhododendron smirnovii* were tested for the existence of alkaloids, anthocyanins, anthraquinones, cardioactive glycosides, coumarins, cyanogenetic glycosides, flavonoids, saponins, reducing sugars, and tannins according to usual methods (25, 26).

The percentages of loss on drying, total ash and acid-insoluble ash were calculated according to methods described in the British Pharmacopoeia 1999, United States Pharmacopoeia 1995, respectively.

### **Preparation of extracts**

Leaf and stem of the plant were separated and dried at room temperature. The dried materials were ground to fine powder. Leaf (10 g) and stem (10 g) were extracted successively in methanol. Maceration was carried out for three days at room temperature. The solvent of each extracted material was removed under reduced pressure.

### **Antimicrobial activity**

The crude extracts obtained from the leaf and stem of *R. smirnovii* were studied for antimicrobial activity. The following microorganisms were used for antimicrobial activity test: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853; *Candida albicans* ATCC 90028, *C.glabrata* ATCC 32554, *C.crusei* ATCC 6258; *C.parapsilosis* ATCC 90019. Antimicrobial activity was evaluated by filter disc diffusion method (27) and the minimal inhibition concentration (MIC) were calculated using broth microdilution method suggested by National Committee for Clinical Laboratory Standards, 1993 (28).

Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) was used when testing bacterial strains. For *Candida* species, Sabouraud Dextrose broth (Difco, USA) was used. The inoculum density was  $1 \times 10^6$  cfu/ml for bacteria and fungi. The extracts were dissolved in 100% dimethylsulfoxide (DMSO). The solutions in the test medium were furnished the required concentration ranging from 1024-0.5 µg/ml. The microtiter plates were incubated at 35°C and read visually after 24 hrs. For *Candida* species, incubation period was 48 hrs. The minimum inhibitory concentration (MIC) values were recorded as the lowest concentrations of the substances that had

no visible turbidity. For bacteria amikacin was used as a positive control; in the case of yeasts, flucanazole was used.

## RESULTS

### Anatomy of the leaf blade

When cross sections are observed under low magnification, leaf is bifacial. Lower surface of leaf is densely covered with trichomes, but covering hairs are fewer on upper surface. Narrow bands of sclerenchyma extend to the upper epidermis are present above each secondary vascular bundle. Indentations are seen along lower surface of the leaf in this area (Fig. 1A). The following anatomical structures and features are observed in cross section of the leaf of *R. smirnovii* (Fig. 1B).

Upper epidermal cells are 2 layered, generally rectangular shaped, small with thin walls. Uppermost epidermis layer is covered with a cuticle and rarely carry dendroit trichomes. Second epidermis layer cells are larger than the first epidermal cells. Beneath this layer there are 4-5 layers of palisade parenchyma. This parenchyma constitutes more or less half of mesophyll and rarely contains druses, but this parenchyma does not contain starch. These regularly arranged parenchyma cells are long and cylindrical with narrow intercellular gaps among them. There are sclerenchymatic bundles composed of 2-3 cells passing through the palisade layer in the part of secondary vascular bundles. These bundles start beneath the first layer of palisade parenchyma and continue towards xylem as a thin-band. Spongy parenchyma cells are usually isodiametric and contain druses and starch granules. These cells have very large intercellular gaps. Secondary vascular bundles are located in spongy parenchyma regularly. Spongy parenchyma beneath these bundles have less intercellular gaps up to lower epidermis. Lower epidermis is composed of one layer with usually rectangular-shaped cells. This epidermis has densely dendroit trichomes and occasionally seen papillae. Base of these dendroit trichomes are usually composed of several layers of cells in two rows. Following this base, there is a long stalk; this stalk is divided into 7-11 cells. Also some dendroit trichomes do not have a stalk and divided just after the base. These two types covering trichomes are densely seen along the whole lower epidermis (Figs 1-5).

### Anatomy of the midrib

In the upper surface midrib is slightly convex, and in the lower surface, it is big and concave. The following anatomic structures are observed in cross section of the midrib (Figs 6-7).

Upper epidermis is composed of two layers of square-shaped cells similar to the laminal epidermis cells. Beneath the upper epidermis there exist collenchyma with 3-5 layers of small cells.

Palisade parenchyma continue in the whole midrib with 2-3 layers, but it is cut by a few collenchyma cells. Sclerenchyma is observed underneath this cells. Sclerenchyma in this region surrounds the whole vascular bundle as a circumference. Sclerenchyma consists of 1-2 small cells near the upper epidermis while it consists of 5-7 layers with large cells in the lower epidermis. Phloem in here is wide and shallow. Phloem cells are small, irregular shaped. Beneath the phloem sclerenchyma is repeated and surrounds the xylem forming a heart-shaped structure. Xylem is divided into two parts by sclerenchyma existing in the center of xylem. Upper xylem layer is wide and shallow but lower xylem is large and concave. Phloem existing as a thin layer above the upper part of xylem, continues by thickening downwards along the concave region. Outside of the sclerenchyma is surrounded by one-layered parenchyma cells with regular arrangement. There exists a loose parenchyma with large intercellular gaps underneath the vascular bundle. These cells become denser while approaching the lower epidermis. 2-3 layers collenchyma follows the parenchyma and extends along the dorsal extrusion. These cells are surrounded by one layered small epidermal cells. Lower epidermis is densely covered with dendroit trichomes.

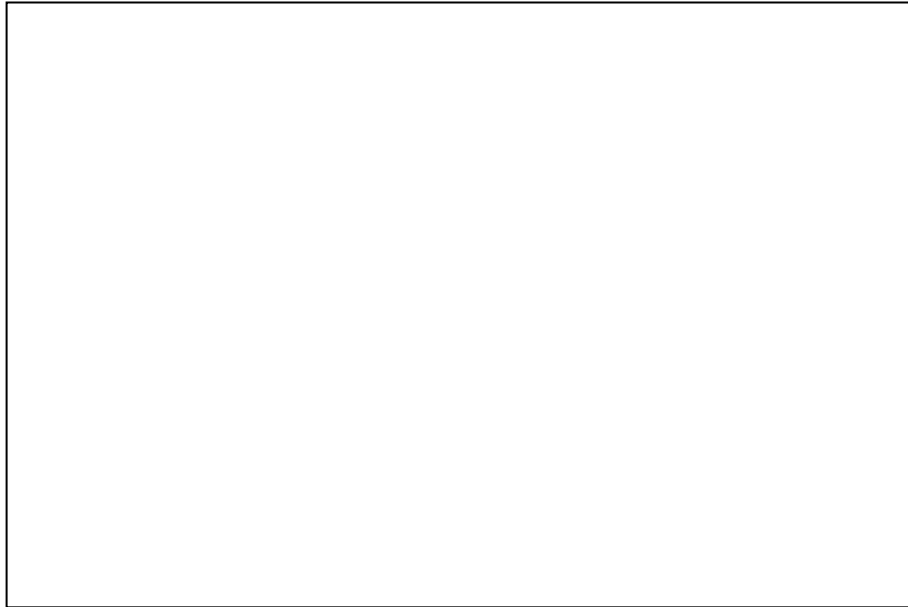
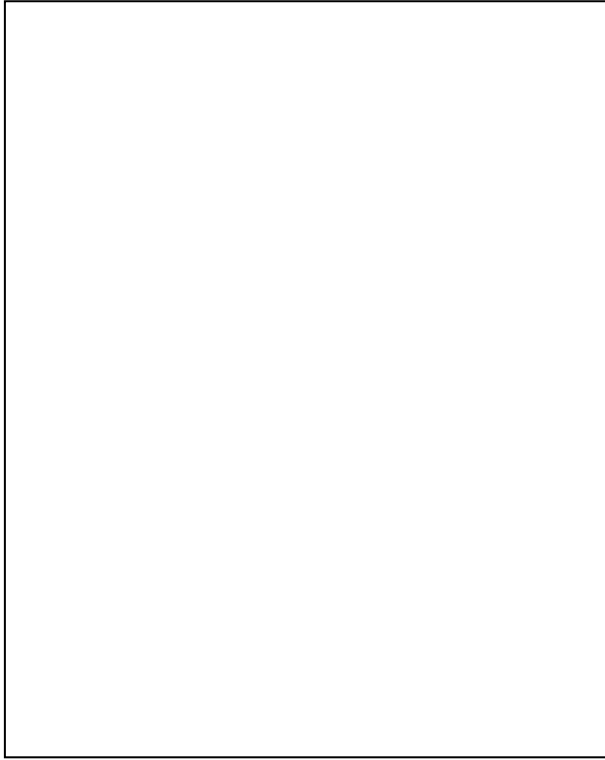
#### **Anatomy of the branch**

When cross section of the branch is studied, following layers are seen : epidermal cells consist of one layer, usually rectangular and their surface is covered by a thick cuticle. There are dendroit trichomes covering the epidermis. Underneath the epidermis circular shaped; 3-4 layers of cortex parenchyma cells are arranged paralel to epidermis. This first layer of cortex parenchyma do not have intercellular gaps. Beneath these cells a thick layer of cortex parenchyma is present with very large intercellular gaps. Cortex parenchyma contains druses abundantly. Beneath the cortex parenchyma, sclerenchyma exist in groups surrounding the phloem that consists of thin walled, irregular shaped small cells. Cambium cells are not clear, thin and generally collapsed and interrupted by pith rays. Pith rays are more frequent. Xylem is 4-5 times larger than phloem. Pith is very large and exists beneath the xylem. Pith cells are circular or oval shaped; furthermore, these cells have large intercellular gaps and contains druses abundantly (Figs 8-10).

#### **Phytochemical studies**

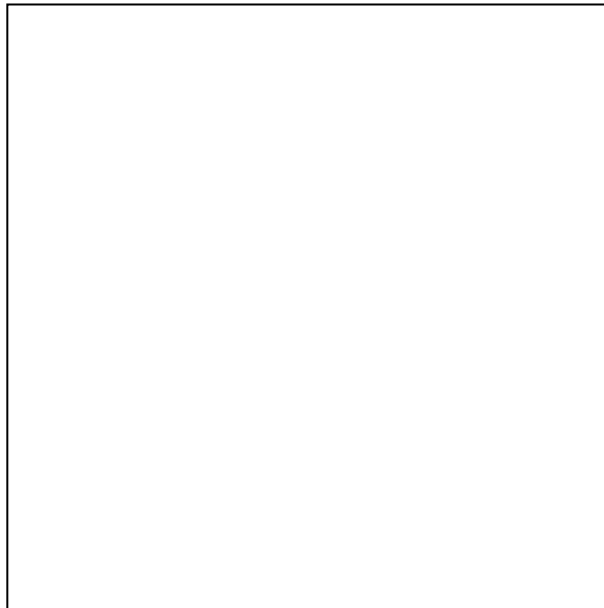
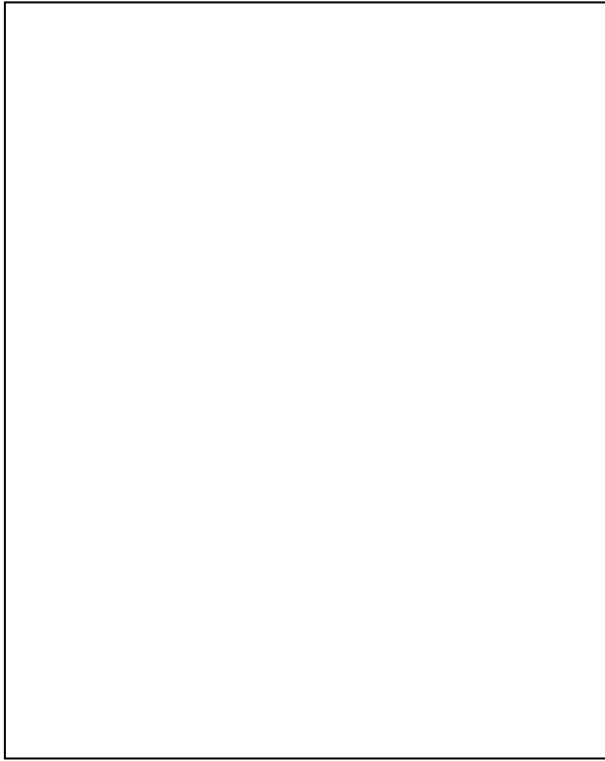
The percentages of loss on drying, total ash and acid-insoluble ash were calculated. The existence of alkaloids, anthocyanins, anthraquinones, cardioactive heterosides, coumarins, cyanogenetic heterosides, flavonoids, saponins, reducing sugars, and tannins were determined in the various extractives and the results are presented in Table 1. With standard chemical tests, the presence of flavonoids, tannins and reducing sugars were detected in the leaf and stem of *R.smirnovii*.

**Figure 1:** *R. Smirnovii* – cross section of leaf blade **A.** General view, **B.** Anatomic properties, **1-** cuticle, **2-** glandular trichomes, **3-** epidermis, **4-** palisade parenchyma, **5-** spongy parenchyma, **6-** druses, **7-** sclerenchyma, **8-** xylem, **9-**phloem, **10-** intercellular space, **11-** lower epidermis.

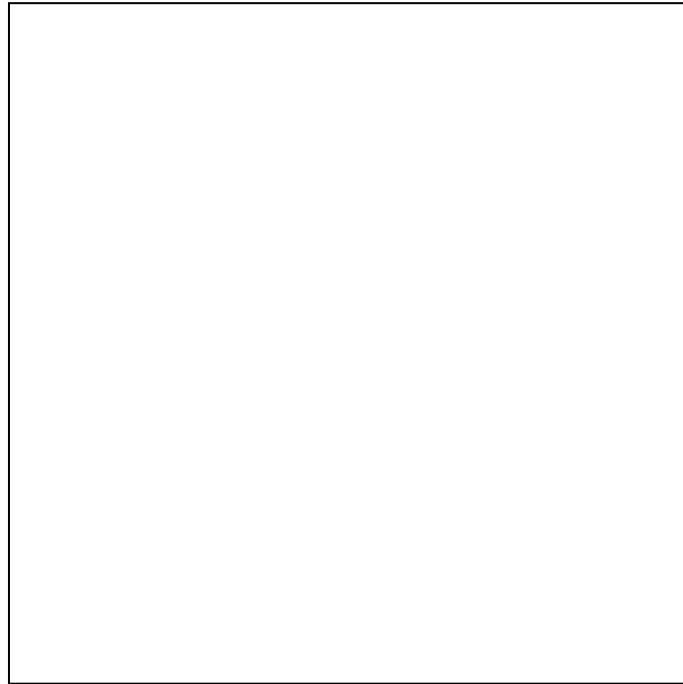
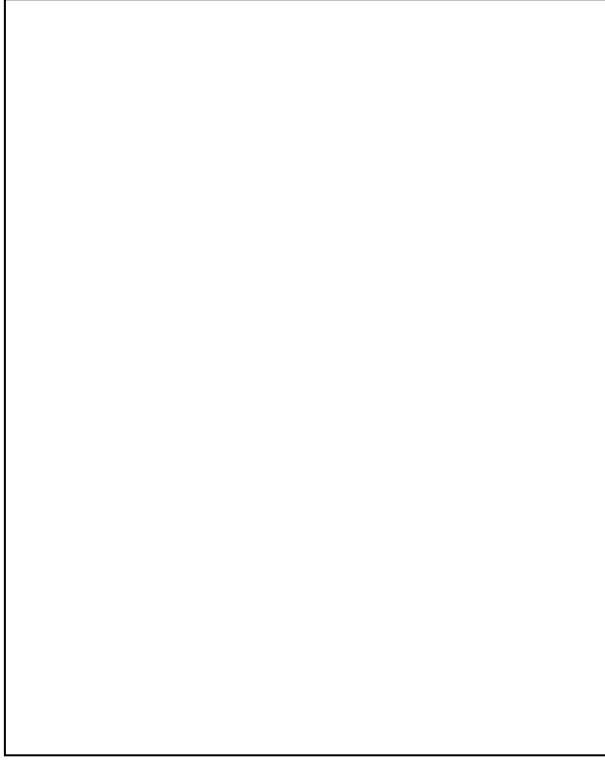


**Figures 2-3.** *R. Smirnovii* – cross section of leaf blade, 1- cuticle, 2- epidermis, 3- palisade parenchyma, 4- spongy parenchyma, 5- druses, 6- starch grain.





**Figures 4-5.** *R. Smirnovii* – cross section of leaf blade. **1-** eglandular trichomes, **2-** epidermis, **3-** papillae, **4-** druses.



**Figures 6-7.** *R. Smirnovii* – cross section of midrib, 1- parenchyma, 2- starch grain, 3- xylem, 4- sclerenchyma, 5- phloem, 6- lower epidermis, 7- eglanular trichomes.

**Table 1.** The major groups of compounds and the percentages of total ash, acid-insoluble ash, loss on drying in the leaf and stems of *R.smirnovii*.

	standard chemical tests	results	
		leaves	stems
Alkaloids	(Mayer, Dragendorff)	-----	----
Anthocyanins	(acid-alkali)	-----	----
Anthraquinones	(Borntrager)	-----	----
Cardioactive heterosides	(Keller-Kliani, Baljet, Lieberman-Burchard)	-----	----
Coumarins	(UV with alkali)	-----	----
Cyanogeneticheterosides	(sodium picrate)	-----	----
Flavonoids	(Cyanidin, ferric chloride)	+	+
Saponins	(Salkowski)	-----	----
Reducing sugars	( Fehling A-B)	+	+
Tannins	(Stiasny, gelatine-salt block, ferric chloride)	+	+
Loss on drying	( USP XXII)	6.4*	7.5*
Total ash	(BP 1999)	3.1*	2.5*
Acid-insol.ash	(USP XXII)	0.12*	0.03*

(+) present; (-) absent, \* percentage (%)

### Antimicrobial activity

The crude methanol extracts obtained from the leaf and stem of *R. smirnovii* were studied for antimicrobial activity. No inhibition zones are seen against the bacteria and the fungi investigated in the stem methanol extract of *R.smirnovii*. DMSO also had no effect on the growth of any of the nine microorganisms. The inhibitory effect of the leaf extract of *R.smirnovii* on microorganisms is summarized in Table 2. The leaf extract of *R.smirnovii* showed antibacterial activity against *E.coli*, *P. aeruginosa*, *S.aureus*, *S.epidermidis*, *E.faecalis* at a concentration of 500 µg / disc with an inhibition zone of 17, 16, 16, 15 and 15 mm, respectively. However, the activity was low compared with amikacin.

**Table 2.** Antimicrobial activity of the methanol extract of the leaf of *Rhododendron smirnovii*.

Leaf Extract	Zones of Inhibition ( mean (mm) ±SEM )*				
	100 µg/ml	250 µg/ml	500 µg/ml	Amikacin (30 µg/ml)	Flucanazole (30 µg/ml)
<b>Gram(+) bacteria</b>					
<i>S.aureus</i>	8.5±0.34	11.5±0.62	16.1±0.91	22.4±0.69	NI**
<i>S.epidermidis</i>	NI*	9.0±0.89	15.3±1.23	28.1±0.70	NI
<i>E.faecalis</i>	NI	10.2±0.48	15.4±1.36	19.3±0.61	NI
<b>Gram(-) bacteria</b>					
<i>E.coli</i>	4.0±0.37	12.1±0.4	17.4±2.12	25.0±0.85	NI
<i>P.aeruginosa</i>	NI	10.0±0.9	16.4±1.48	23.2±0.87	NI
<b>Fungi</b>					
<i>C.albicans</i>	NI	NI	14.2±1.11	NI	21.4±0.92
<i>C.krusei</i>	NI	NI	11.9±0.40	NI	20.4±1.05
<i>C.glabrata</i>	NI	NI	15.2±0.86	NI	27.2±0.59
<i>C.parapsilosis</i>	NI	NI	12.0±0.42	NI	19.5±1.05

\* Results are the mean of four determinations ± S.E.M expressed as diameter of zone of inhibition in mm.,

\*\* NI: No inhibition zone

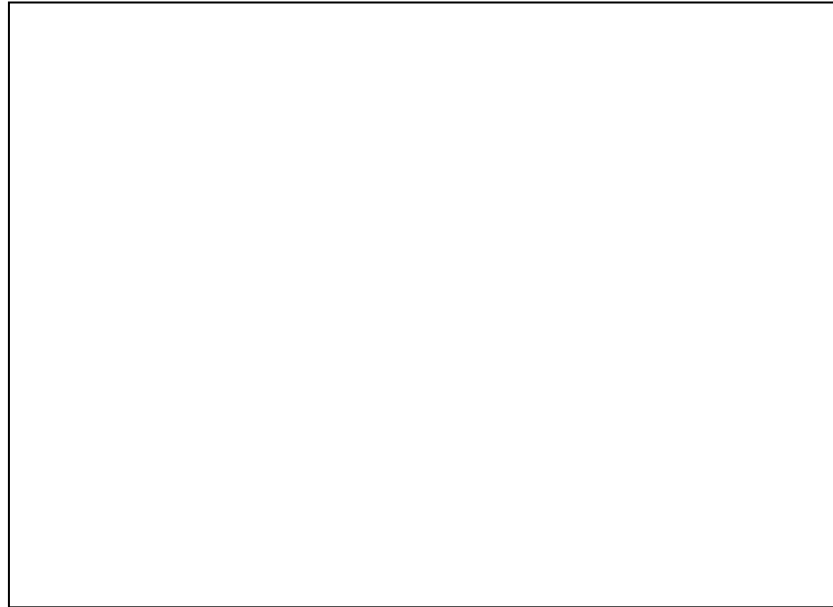
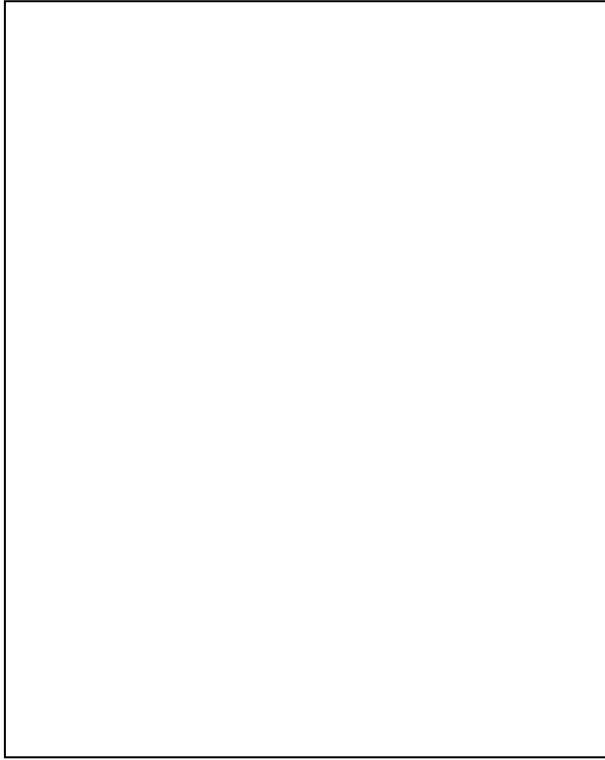
The lowest MIC value was obtained for the extract of *R.smirnovii* against *S.aureus* and *P.aeruginosa* with MIC value of 32 µg / ml of two microorganisms (Table 3). Susceptibility of *S.aureus* and *P.aeruginosa* to amikacin was between 2 and 4 µg / ml . The MIC of *R.smirnovii* leaf extract against *C. glabrata* was 128 µg / ml. The leaf extract of *R.smirnovii* was found to have lower activity against yeasts (*C. albicans*, *C. crusei* and *C. parapsilosis* ) at a concentration of 256 µg / ml . As a result, *R.smirnovii* has antimicrobial activity against some Gram (+) and Gram (-) bacteria. The extract showed more antibacterial activities than antifungal activities.

**Table 3.** The MIC of *Rhododendron smirnovii* leaf extract (µg/ml).

	Leaf Extract	Amikacin	Flucanazole	DMSO
<b>Gram(+) bacteria</b>				
<i>S.aureus</i>	32.4±1.16	4.2±0.16	NI	NI
<i>S.epidermidis</i>	64.0±9.23	4.1±0.10	NI	NI
<i>E.faecalis</i>	64.0±14.31	1.1±0.14	NI	NI
<b>Gram(-) bacteria</b>				
<i>E.coli</i>	64.01±0.47	32.0±0.65	NI	NI
<i>P.aeruginosa</i>	32.2±1.07	2.5±0.32	NI	NI
<b>Fungi</b>				
<i>C.albicans</i>	256.0±27.93	NI	0.50±0.11	NI
<i>C.krusei</i>	256.0±24.19	NI	32.0±1.13	NI
<i>C.glabrata</i>	128.0±16.52	NI	4.1±0.10	NI
<i>C.paraphilosis</i>	256.0±26.98	NI	2.3±0.30	NI

\* NI: No inhibition zone

**Figure 8.** *R. Smirnovii* – cross section of the branch, **A.** General view, **B.** Anatomic properties, **1-** epidermis, **2-** eglandulartrichomes, **3-**cortex parenchyma, **4-** sclerenchyma, **5-** phloem, **6-** cambium, **7-** xylem, **8-** pith ray, **9-** pith, **10-** druses.



**Figures 9-10.** *r. smirviovii* – cross section of the branch, **1-** cuticle, **2-** epidermis, **3-** glandular trichomes, **4-** cortex parenchyma, **5-** intercellular space, **6-** sclerenchyma, **7-** phloem, **8-** cambium, **9-** xylem, **10-** pith ray, **11-** druses.

## DISCUSSION

The present study, showed that anatomical characters such as the dendroid trichomes, papillose epidermis and types of crystals have taxonomic value. Metcalfe & Chalk (1965) pointed out that the lower epidermis contain papillae in Ericaceae and solitary crystals generally present in the lamina of some Ericaceae plant (29). In our research concerning this feature, there was papillae in the lower epidermis of the leaf of *R.smirnovii*. In the spongy parenchyma cells of the leaf, cortex parenchyma and pith of the stem contain druses as crystals.

Metcalfe & Chalk (1965) reported the leaf structure of some *Rhododendron* species. They described the occurrence of different kinds of trichomes in some species of the genus (29). Chamberlain (1982) gave information about taxonomic characters of *Rhododendron* genus. He remarked that the leaf indumentum (or lack of it) is the most valuable taxonomic character (4). In our study, it was observed that *R.smirnovii* had densely dendroid trichomes on the lower epidermis of the leaf and epidermis of the stem. There was two types of trichomes; 1) with a long stalk, and this stalk divided into 7-11 cells 2) without stalk and divided just after the base.

The chemical constituents from different species of genus *Rhododendron* had been isolated and characterized in the past. The occurrence of simple phenols and phenolic acids, flavonoids, tannins, diterpenes, triterpenes, and essential oils have been reported (10-14). The present study revealed that the leaf and stem of *R. smirnovii* contain flavonoids, tannins and reducing sugars as the major groups of constituents.

Tasdemir et al.(2004) reported the middle polarity extracts of the leaves of *R.smirnovii* was particularly active against Gram (+) bacteria tested (23). The present study shows that the leaf methanol extract of *R.smirnovii* has antimicrobial activity against some Gram (+) and Gram(-) bacteria. The extract showed more antibacterial activities than antifungal activities. The stem methanol extract of *R.smirnovii* did not show any antimicrobial activity against the bacteria and the fungi investigated in this study. In conclusion, in this study *R.smirnovii* has been investigated in details in terms of leaf and stem anatomy, major groups of chemical compounds and antimicrobial activity.

### Acknowledgements

The authors thank Assist.Prof. Salih Terzioğlu (Black Sea Technical University, Faculty of Forestry, Trabzon, Turkey) for his help in the field work.

**REFERENCES**

1. **Heywood, V.H.** "Flowering plants of the world" Oxford University Press, Oxford (1978)
2. **Terzioğlu, S., Merev, N., Anşin, R.** "A Study on Turkish *Rhododendron* L.(*Ericaceae*)"  
*Turk J. Agric. For.* 25, 311-317 (2001)
3. **Chamberlain, D., Tian-Lu, M.** "The distribution of *Rhododendron* subgenus *hymenanthes* with special reference to yunnan (W China)" *Notes RBG Edinb.* 43 (1): 7-13 (1985)
4. **Chamberlain, D.F.** "A revision of *Rhododendron* II. Subgenus *Hymenanthes*" *Notes RBG Edinb.* 39(2): 209-486 (1982)
5. **Chamberlain, D., Hyam, R., Argent, G., Fairweather, G., Walter, K.S.** "The genus *Rhododendron*: Its classification and synonymy" Royal Botanic Garden Edinburgh, Edinburgh (1996)\*\*\*
6. **Chamberlain, D., Hyam, R.** "The genus *Rhododendron*: a case study to test the value of various molecular techniques as measures of biodiversity" In: *Molecular Tools for Screening Biodiversity: Plants and animals* ( eds Karp, A., P.G. Isaac, D.S. Ingram). 441-448 pp, Chapman and Hall, London (1998)
7. **Merev, N., Yavuz, H.** "Ecological wood anatomy of Turkish *Rhododendron* L. ( *Ericaceae*). Intraspecific variation" *Turk J Bot.*, 24: 227-237 (2000)
8. **Sütlüođlu, N, Mat, A., Satganođlu, Y.** "Poisoning by toxic honey in Turkey" *Arch.Toxicol.*, 67: 148-150 (1993)
9. **Baytop, T.**, Therapy with Medicinal Plants in Turkey ( Past and Present). 2nd ed. Nobel Tıp Kitapevleri, Istanbul, Turkey. pp. 161, 275 (1999)
10. **Hegnauer, R.** Chemotaxonomie der Pflanzen . Vol.4, Birkhauser Verlag, Basel, p.65-93 (1966)
11. **Harborne, J.B., Williams, C.A.** "Leaf Survey of Flavonoids and Simple Phenols in the genus *Rhododendron*" *Phytochemistry* , 9: 875-879 (1971).
12. **Harborne, J.B.** " Flavonoid Patterns and phytogeography.The Genus *Rhododendron* Section *Vireya*." *Phytochemistry*, 25 (7): 1641-1643 (1986)
13. **Doss, R.P., Hatheway, W.H., Hrutfiord, B.F.** "Composition of Essential Oils of Some Lepidote *Rhododendron*" *Phytochemistry* 25(7): 1637-1640 (1986)



14. **Dobhal, M.P., Joshi, Y.C., Joshi, P. A** “Review on Genus *Rhododendron*” *Herba Polonica* 37 (2): 89-94 (1991)
15. **Thapliyal, R.P., Bahuguna R.P.** “Constituents of *Rhododendron barbatum*” *Fitoterapia* LXIV(5): 475 (1993)
16. **Belousov, M.V., Berezovskaya, T.P., Komissarenko, N.F., Tikhonova, L.A.** “Flavonoids of Siberian–far-eastern species of *Rhododendrons* of the subspecies *Rhodorastrum*” *Chem.Nat.Comp.* 34 (4): 510-511 (1998)
17. **Swiderski, A., Muras, P., Koloczek, H.** “Flavonoid composition in frost-resistant *Rhododendron* cultivars grown in Poland” *Scientia Horticulture* 100: 139-151 (2004)
18. **Stevens, P.F.** *Rhododendron* L. In: *Flora of Turkey and the East Aegean Islands*, Eds.:P.H. Davis, Vol. 6, Edinburgh University Press, 90-94 pp (1978)
19. **Güner, A, Özhatay, N., Ekim, T., Başer, K.H.C.** *Flora of Turkey and the East Aegean Islands*, Vol.11: 181-183, Suppl.2, Edinburgh University Press (2000)
20. **Tasdemir, D., Demirci, B., Demirci, F., Dönmez, A.A., Baser, K.H.C., Ruedi, P.** “Analysis of the volatile components of five Turkish *Rhododendron* species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS )” *Z.Naturforsch. ( C )* 58 (11-12 ): 797-803 (2003)
21. **Erdemoğlu, N., Küpeli, E., Yesilada, E.** “Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine” *J. Ethnopharm.* 89: 123-129 (2003)
22. **Orhan, I., Şener, B., Choudhary, M.I., Khalid, A.** “Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants” *J. Ethnopharm.* 91: 57-60 (2004)
23. **Tasdemir, D., Dönmez, A.A., Çalı, I., Ruedi, P.** “Evaluation of biological activity of Turkish plants. Rapid screening for the antimicrobial, antioxidant, and acetylcholinesterase inhibitory potential by TLC bioautographic methods” *J.Pharm.Biol.* 42 ( 4-5), 374-383 (2004)
24. **Çelebioğlu, S., Baytop, T.** “A New Reagent for Microscopic Investigation of Plant. Publication of the Institute of Pharmacognosy” No.10, 19:301, Istanbul (1949)
25. **Farnsworth, N.R.** “Biological and phytochemical screening of plants” *J.Pharm. Sci.* 55: 225-276 (1966)

26. **Sakar, M.K., Tanker, M.** Phytochemical analysis ( determination, quantitative analysis and isolation ), Publications of the Ankara University, Faculty Pharmacy, Publication no: 67, Ankara (1991)
27. **Quinin, P.J., Carter, M.E., Markey, B.K., Carter, G.R.** Clinical veterinary microbiology. Wolfe Pub.Spain, Year Book Europe Ltd. ISBN 07234 (1994)
28. Anonymus, National Committe for Clinical Laboratory Standards Methods for dilution antimicrobial susceptibility test. Approved Standard M2-A5. National Committe for Clinical Laboratory standards, Villanova, P.A (1993)
29. **Metcalfe, C.R., Chalk, L.** Anatomy of Dicotyledons 2, Clarendon Pres. Oxford 827-836 (1965)

Recived: 20.07.2005

Accepted: 28.11.2005