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RESEARCH ARTICLE

Morphologic, Anatomical, and Chromatographic Studies on *Eucalyptus* (L'Hér.) Samples from the Market

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Summary

Eucalyptus (L'Hér.) species (Myrtaceae) are known as "okaliptus agaci" or "sitma agaci (malaria tree) " in Turkey. Eucalyptus genus is indigenous to Australia and represented by up to 700 species. It forms small forests in some regions of Anatolia. Decoction of E. camaldulensis leaves is used for sinusitis, bronchitis and toothache in Turkey as folk medicine. The aim of this study is to identify the species of the Eucalyptus leaves sold in the Turkish market, named as okaliptus. Morphological, anatomical examinations and thin layer chromatographic analyses were used to analyse the samples collected from the Turkish market. For this purpose, 10 samples were collected from different herbal shops in Ankara, Istanbul and Konya, and compared with E. camaldulensis Dehnh., E. globulus Labill., and E. grandis Hill Ex Maiden leaves collected from the Eucalyptetum in Karabucak (Tarsus, Mersin). Additionally 3 essential oil samples were purchased from herbal shops, their thin layer chromatography profiles were also compared with the essential oils obtained from Eucalyptus camaldulensis, E. globulus, and E. grandis leaves. According to the results, all the leaf samples sold in the market as E. globulus, were determined as E. camaldulensis and adulterations were detected in essential oil samples.

Key Words: *Anatomy, Eucalyptus, morphology, Myrtaceae, essential oil, adulteration.*

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Piyasadan Toplanan Eucalyptus (L'Hér.) Örnekleri Üzerinde Anatomik, Morfolojik ve Kromatografik Çalışmalar

Özet

Eucalyptus (L'Hér.) türleri (Myrtaceae), ülkemizde "ökaliptus, sıtma ağacı" olarak bilinmektedir. Vatanı Avustralya olan bitki, dünya üzerinde 700 kadar tür ile temsil edilmektedir. Eucalyptus türleri Anadolu'nun bazı bölgelerinde küçük ormanlar halinde bulunmaktadır Türkiye'de ökaliptus yapraklarının dekoksiyonu, halk ilacı olarak, sinüzit, bronşit ve diş ağrısında kullanılmaktadır. Çalışmamızda, Türkiye piyasasında ökaliptus adıyla satılan Eucalyptus yapraklarının türlerinin teşhis edilmesi amaçlanmıştır. Piyasadan toplanan örnekler, morfolojik, anatomik incelemeler ve ince tabaka kromatografisi analizi kullanılarak ile araştırılmıştır. Bu amaçla Ankara, Mersin ve Konya'daki farklı aktarlardan toplanan 10 örnek, Tarsus Karabucak ökaliptüs ormanından toplanan E. camaldulensis Dehnh., E. globulus Labill. ve E. grandis Hill Ex türlerine ait yapraklar ile karşılaştırılmıştır. Ayrıca, aktarlardan alınan üç farklı ökaliptus yağı örneğinin ince tabaka kromatografisi profilleri de Eucalyptus camaldulensis, E. globulus ve E. grandis yapraklarından elde edilen uçucu yağlar ile karşılaştırılmıştır. Yapılan incelemeler sonucunda; ofisinal tür olan E. globulus'a ait olduğu belirtilerek satılan tüm yaprak numunelerinin E. camaldulensis'e ait olduğu, uçucu yağ örneklerinde ise tağşişat yapıldığı tespit edilmiştir.

Anahtar Kelimeler: *Anatomi, Eucalyptus, morfoloji, Myrtaceae, ökaliptus uçucu yağı, tağşiş*

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This study was a part of Ayşe Gül Tombul's M.Sc. thesis called "*Eucalyptus*' of Turkey"
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INTRODUCTION

Eucalyptus (L'Hér.) (Myrtaceae) is a large genus of evergreen trees that includes more than 700 species. It is one of the world's most widely planted genera, native to Australia. It has been grown in many countries, as well as in Turkey since 1885 (1, 2). *Eucalyptus bicostata* Maiden, Blakely & Simmonds, *E. blakelyi* Maiden, *E. botryoides* Sm., *E. camaldulensis* Dehnh., *E. cinerea* Benth., *E. citriodora* Hook., *E. dumosa* J. Oxley, *E. dwyeri* Maiden & Blakely, *E. globulus* Labill., *E. grandis* Hill Ex Maiden, *E. leucoxylon* F. Muell., *E. macarthuri* Deane et Maid., *E. maidenii* F. Muell., *E. oleosa* F. Muell. Ex Miq., *E. sideroxylon* (A. Cunn.) Benth., *E. smithii* R.T. Baker, *E. tereticornis* Sm., *E. viminalis* Labill. and *E. viridis* R.T. Baker are the most common species planted in the eucalyptetums in the South and Southeast regions of Turkey (2).

Wood, leaf and essential oil of *Eucalyptus* species are used for many purposes in pharmaceutical and cosmetics industries. Wood of the tree is mainly used for manufacturing paper, fiberboard, charcoal, acetic acid, methanol and cellulose (3). In traditional medicine, *Eucalyptus* leaves are used as antiseptic against infections of the upper respiratory tract such as common cold, influenza, bronchitis and sinusitis. Additionally its essential oil has a therapeutic application in treatment of pulmonary infections by inhalation and is added to herbal candies, pastilles and cough syrups as antiseptic (4, 5).

Medicinal uses of *Eucalyptus globulus* leaf and essential oil are described in the WHO Monographs on Selected Medicinal Plants, PDR for Herbal Medicines, ESCOP Monographs, German Commission E Monographs (6-9). Additionally, *E. globulus* is mentioned as the officinal species in the European Pharmacopoeia as well (10).

Eucalyptus leaves are collected in different regions of Turkey. Both leaves and the essential oils of the leaves are sold for various purposes in the market. The aim of this study is to investigate the morphological and anatomical characteristics of *Eucalyptus* leaf samples purchased from different herbal shops and to compare their characteristics with standard samples collected from Tarsus (11). Additionally, thin layer chromatography (TLC) method was used to compare the TLC profile of the extracts and essential oil samples with the reference extracts and essential oils obtained from *E. camaldulensis* Dehnh., *E. globulus* Labill., and *E. grandis* Hill Ex Maiden leaves.

MATERIAL AND METHODS

Plant Material

Ten *Eucalyptus* leaf samples were purchased from different herbal shops in Mersin, Konya and Ankara in 2008 (Table 1). Additionally 3 *Eucalyptus* essential oil samples were bought from Konya. *E. camaldulensis*

Table 1. Plant materials of *Eucalyptus* species.

Sample Name	Sample Code	Collection Date	Place
Leaves	S1	20.11.2008	Kızılay/ Ankara
	S2	20.11.2008	Ulus/ Ankara
	S3	20.11.2008	Ulus/ Ankara
	S4	20.11.2008	Ulus/ Ankara
	S5	20.10.2008	Selçuklu/ Konya
	S6	24.11.2008	Karatay/ Konya
	S7	24.11.2008	Karatay/ Konya
	S8	22.11.2008	City Centre/ Mersin
	S9	22.11.2008	City Centre/ Mersin
	S10	22.11.2008	City Centre/ Mersin
Essential Oils	VO 1	04.10.2008	Meram/ Konya
	VO 2	13.10.2008	Karatay/ Konya
	VO 3	20.10.2008	Selçuklu/ Konya
References	*E. camaldulensis* Dehnh. (EC)	22.11.2008	Tarsus/ Mersin
	E. globulus Labill. (EGL)	22.11.2008	Tarsus/ Mersin
	E. grandis W. Hill ex Maiden (EGR)	22.11.2008	Tarsus/ Mersin

Dehnh., *E. globulus* Labill., and *E. grandis* Hill Ex Maiden were collected as reference samples from Tarsus Karabucak Eucalyptetum (Mersin). Reference samples were dried in the shade and herbarium specimens including branches with buds, fruits, young and mature leaves were prepared and kept in the Herbarium of Gazi University Faculty of Pharmacy.

Morphological Analysis

Dried leaf, flower and buds of the reference species (*E. camaldulensis, E. globulus* and *E. grandis*) and of the market samples were photographed on a scaled paper. Leaves in different sizes and shapes were chosen to make an elaborate comparison. Lamina shape, colour, and dimensions of the leaves and other morphological characters were examined.

Microscopic Analysis

Leaf samples were cut into 3 pieces and stored in water for a while to get to the cross section of the midribs. Cross sections were performed by hand from all references and market samples, and examined under light microscope (Motic BA200) in chloral hydrate solution and Sartur reagent. Sartur reagent contained KI-I, aniline, Sudan III, lactic acid, alcohol and water (12). The cross-sections were photographed by a camera (7.2 megapixels) attached to the microscope.

Essential Oil Content

Leaves of the reference species (*E. camaldulensis, E. globulus* and *E. grandis*) were cut and weighted just before the experiment. The essential oils of the leaves were obtained according to the "Determination of Essential oil in Herbal Drugs (2.8.12) " part and "*Eucalyptus* leaf" monograph in the European Pharmacopoeia 7.0 (10). These essential oils were used as reference in TLC analysis of three oil samples (VO1, VO 2 and VO 3) bought from the herbal shops that are sold as *E. globulus* essential oils.

Thin Layer Chromatography (TLC) Analyses of Essential Oils and Leaf Extracts

TLC analyses were performed according to the related part in the "*Eucalyptus* leaf" and "*Eucalyptus* oil" monographs in EP 7.0 (10). Toluene extracts of the leaf samples (reference and market) were prepared. Dried and powdered leaves (0.5 g) were mixed with toluene (5 ml) in vials. The extraction was completed after storing capped vials in an ultrasonic bath for 5 min. Then, samples were filtered. Essential oil samples obtained from the references (EC, EGL, EGR) and those bought in the market (VO1, VO 2 and VO 3) were diluted with toluene. Both extracts and the essential oils were applied to silica gel TLC plates as bands and eluated with toluene/ethyl acetate (90:10). Anisaldehyde solution was sprayed to air dried plates and the colours were examined after heating at 100°C for 5 minutes. 1,8-cineole was used as a reference compound.

RESULTS AND DISCUSSION

Morphological Analysis

Eucalyptus camaldulensis: Leaves broadly or narrowly lanceolate to falcate, 1-3.5 cm x 6-20 cm. Base cuneate to asymmetric, apex acuminate and narrow, petiole 1-2 cm. Leaf colour is dull blue-green to pale yellow. Venation is pinnate, lateral veins joined near the edge of the leaf and form a parallel vein to the edge. Umbels 2-10 flowered, cream-yellow. Fruit conical, greenish-brown. (Figure 1-EC). Powder of the leaves are very fibrous.

Eucalyptus globulus: Juvenile leaves ovate to orbiculate and broadly lanceolate, 2.5-7.5 cm x 3.5-18 cm. Leaf sesile or with a 1-4 cm bent petiole. Leaf acuminate or mucronate at apex, cordat and rarely asymmetric at the base. Leaf surface is waxy and dark green (Figure 1-EGL). Adult leaves lanceolate-falcate, 0.5-3 cm x 3-27 cm with a 0.5-3 cm petiole. Leaf, acuminate at apex, cuneate and rarely asymmetric at the base, venation is prominent and the midrib is yellowish-green. Lateral veins anastamose near the margin to a continouous line. Leaf surface is bright green (Figure 1-EGL). Flowers are solitary in the axils on flattened stalks. They are 4-5.5 cm wide. The fruit is a hard woody capsule, broadly top shaped-globose with a wide flat disk. The fruit is 1.5-2.5 cm across.

Eucalyptus grandis: Leaves wide lanceolate to narrow lanceolate, 2.5-5 cm x 11-21 cm with a 2-4 cm petiole. Leaf acuminate at apex, mostly asymmetric

at the base. Leaf bright dark green. The venation is very prominent and laterals veins are conspicuous, intramarginal vein up to 1 mm from the margin. Umbels 5-10 flowered, short stalked or stalkless. Buds pyriform, pear shaped fruit capsules with short stipes are in groups. Fruits 5-8 mm long and 4-7 mm wide with 4-5 valves. (Figure 1-EGR).

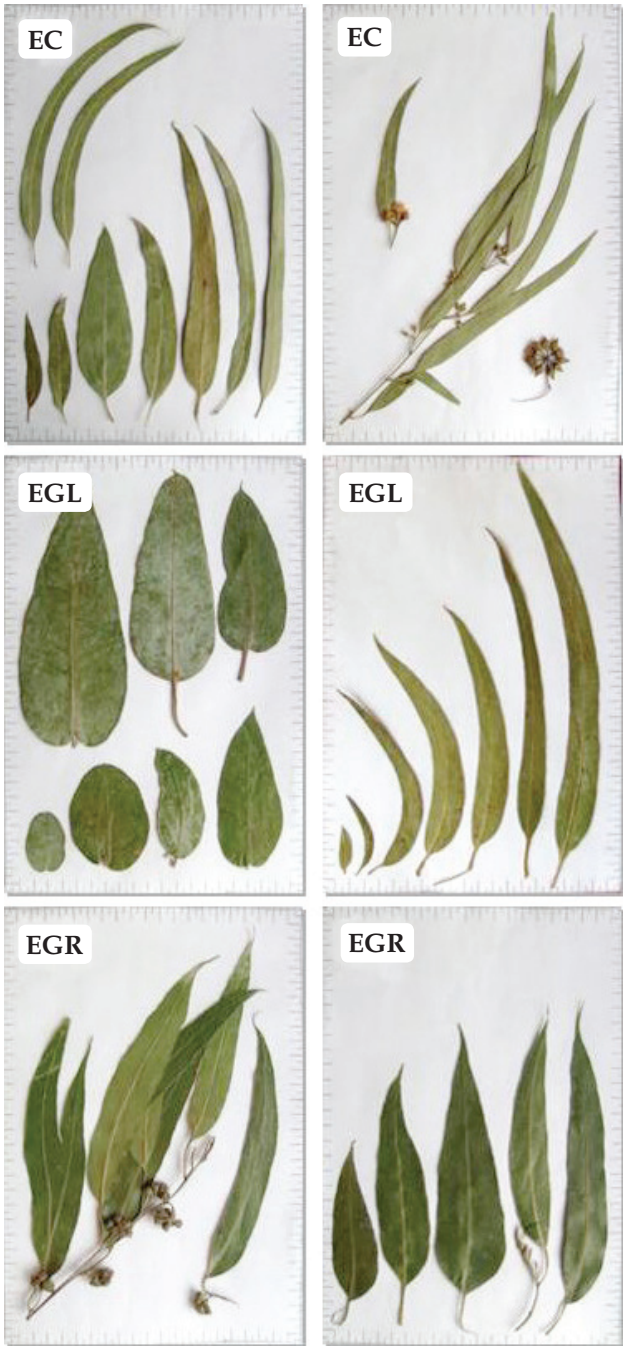

Figure 1. Dried leaf samples of reference species (EC:*E. camaldulensis*, EGL: *E. globulus* and EGR: *E. grandis*)

Market Samples

S1. Leaves lanceolate-broad lanceolate, apex acute-acuminate, cuneate to asymmetrical at the base, 3-11 cm long, 1.5-4 cm wide, pale yellowish-green. Some leaves have warty dark brown spots on both surfaces. Fruit capsules are conical. Many long and short branch pieces were found in the sample. (Figure 2.1).

S2. Leaves lanceolate, rarely falcate, bended backwards, apex acute to acuminate, acute at the base, 5-11 cm long, 1-2.3 cm wide, range-yellowish to green. Some brown leaves and pieces of branches were found in the sample. (Figure 2.2).

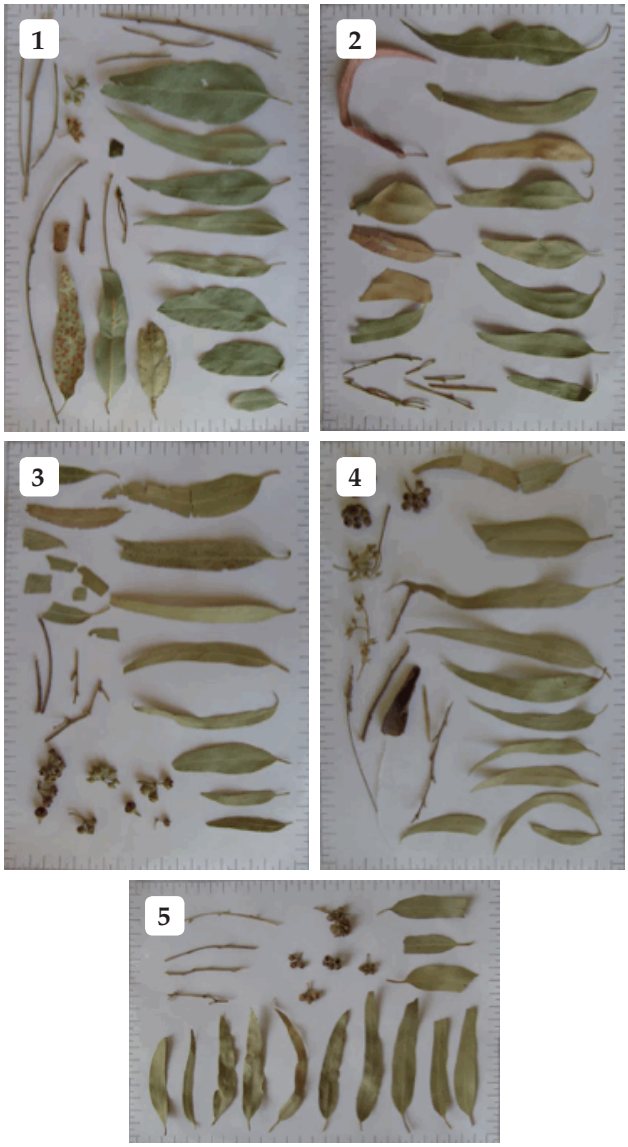

Figure 2. Leaves, flowers, buds, branches and some foreign matters of market samples of *Eucalyptus* species.

S3. Leaves lanceolate, acute to acuminate at the top, cuneate to asymmetrical rounded at the base, 6-14 cm long, 1-2.5 cm wide, pale yellowish-green, fruit capsules are conical (Figure 2.3).

S4. Leaves narrow lanceolate-lanceolate, acuminate at the top, cordate to asymmetrical rounded at the base, 5-15 cm long, 1-3 cm wide, grayish green, many conical fruits and long pieces of branches and foreign materials were found in the sample (Figure 2.4).

S5. Leaves narrow lanceolate-lanceolate, apex acute, acute to asymmetrical rounded at the base, 7-12 cm long, 0.8-2 cm wide, pale green, fruit capsules are conical (Figure 2.5).

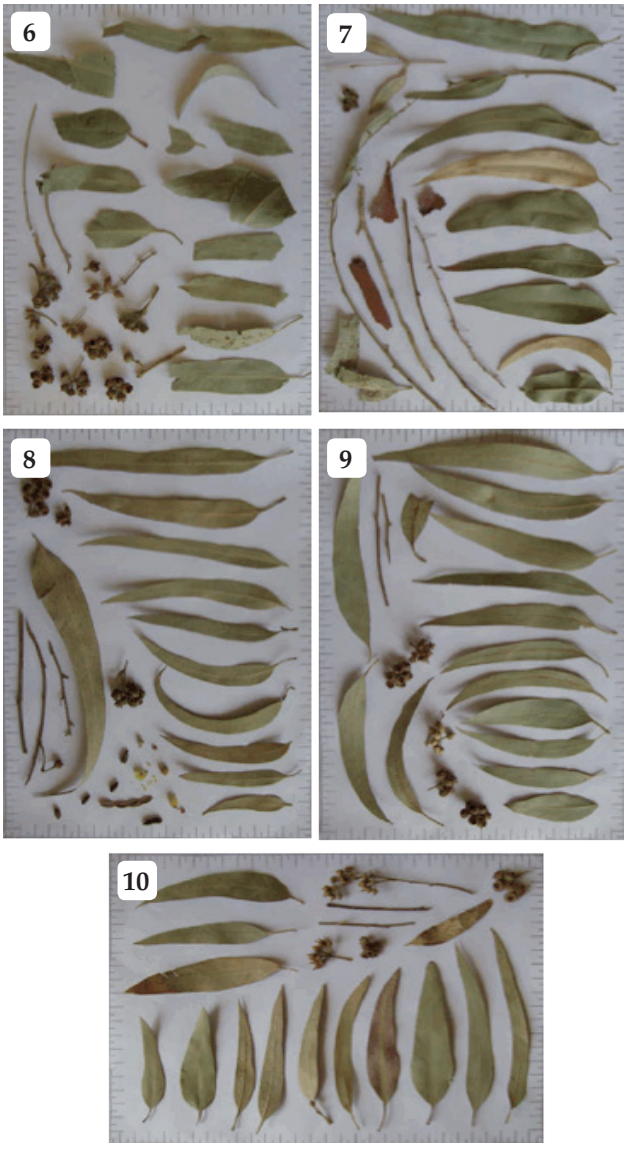

Figure 3. Leaves, flowers, buds, branches and some foreign matters of market samples of *Eucalyptus* species.

S6. Leaves lanceolate-broad lanceolate, rounded-asymmetric at the base leaves are mostly broken, pale green, fruit capsules are conical. Many fruits and some branch pieces were found in the sample (Figure 3.6).

S7. Leaves lanceolate to falcate, apex acute to acuminate, rounded to asymmetrical rounded at the base, 5-16 cm long, 1-2.8 cm wide, bended backwards, dark green to pale green and beige. Long branches and brown bark pieces were found in the sample (Figure 3.7).

S8. Leaves narrow lanceolate to falcate, acuminate at the top, acute to cuneate at the base, 6-20 cm long, 1-2.5 cm wide, grayish green. Conical fruits, branch pieces, some foreign fruit and seeds were found in the sample (Figure 3.8).

S9. Leaves ovate to lanceolate, falcate, acuminate at the top, acute-cuneate at the base, 7-17 cm long, 1.5-2.5 cm wide, bended backwards, pale green, fruit capsules are conical (Figure 3.9).

S10. Leaves ovate to lanceolate, rarely falcate, apex acute to acuminate, cuneate to rounded at the base, 7-15 cm long, 1-2.5 cm wide, beige-yellowish green. Fruits capsules are conical (Figure 3.10).

Microscopic Analysis:

Anatomical characteristics of the leaves of *Eucalyptus* genus are used to differentiate the species. In certain species, oil glands were reported in pith and midrib (13). The degree of ornamentation of waxes on leaf surfaces are frequently correlated with taxonomic groupings of this genus, which also proved useful as indicators of natural groupings of species (14). Thus, sections of all *Eucalyptus* leaf samples were prepared and examined. The examined *Eucalyptus* species presented glabrous and unifacial leaves with stoma appearing at the same level as the epidermal cells.

Eucalyptus camaldulensis: In the cross-section of the *E. camaldulensis* leaf, size of the epidermal cells was

found to be smaller than *E. globulus*, also the outer walls of the epidermal cells were much thicker (Figure 4). There was a thin cuticle layer over the epidermis. Palisade parenchyma was aligned regularly and strictly in tree rows. Sponge parenchyma cells were narrow and contained small intercellular spaces. There were many stomata perforating the surface. Small and circular schizolysigenous oil glands in palisade tissue were few and often close to the upper epidermis (Figure 4). Heart shape midrib protruded from both epidermises equally. Sclerenchyma and vascular bundles were prominent and vascular bundle in the midrib is heart-shaped. There were a few number of calcium oxalate crystals and druses and crystals were not aligned regularly.

Eucalyptus globulus: Epidermal cells of *E. globulus* leaves were greater than *E. camaldulensis* epidermal cells. Lower epidermal cells were slightly curved towards the cuticle. The palisade parenchyma was generally aligned regularly in two rows, rarely in a single row. The spongy parenchyma was a narrow zone of loosely arranged cells between the two palisade parenchyma layers. The parenchyma cells of lower epidermis were cylindrical and longer than the parenchyma cells of upper epidermis (Figure 4). The midrib was fan-shaped and more protruded from the lower epidermis. Fibrovascular bundles, in midrib, interrupted arc of slightly lignified pericyclic fibres that occurred just outside these bundles. Many large schizolysigenous oil glands were located in the mesophyll layer. Calcium oxalate crystals were aligned regularly and many druses were located in the spongy parenchyma.

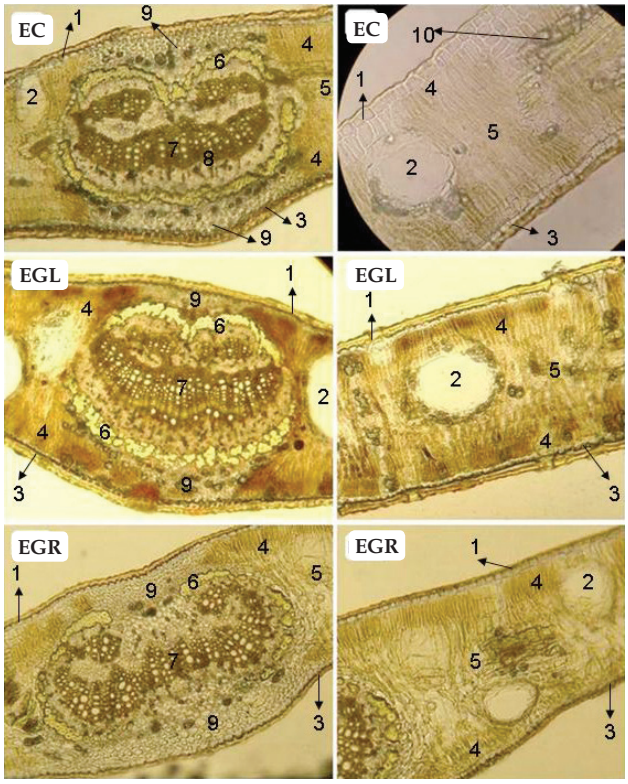

Figure 4. Midrib and leaf blade cross-sections of *E. camaldulensis* (EC), *E. globulus* (EGL) and *E. grandis* (EGR) leaves. (1: Upper epidermis, 2: Oil Gland, 3: Lower epidermis, 4: Palisade parenchyma, 5: Sponge parenchyma, 6: Sclerenchima, 7: Xylem, 8: Phloem, 9: Collenchyma, 10: Calcium oxalate crystals)

Eucalyptus grandis: The sponge parenchyma cells of *E. grandis* leaves were very loose and intercellular spaces were wide (Figure 4). The fibrovascular bundles in midrib throughout the spongy parenchyma, occupied a large area. The midrib was almost flat in the upper epidermis and slightly protruded from the lower epidermis. The sclerenchyma bundles surrounding the xylem and phloem in the midrib were thinner than other two *Eucalyptus* species. Vascular bundles were more elliptical and flattened and the midrib vascular bundle was in the shape of flat-arc. In the midrib, the subepidermal collenchyma appeared as two caps turned towards the two leaf surfaces. Many schizolysigenous oil glands were spread in the mesophyll layer.

Market Samples: According to the microscopic analysis of the samples that are sold as *E. globulus* in herbal shops, it was seen that the midribs were protruded from both epidermis layers equally (Figure 5). In addition, the midrib was surrounded by many sclerenchyma bundles (Figure 5). Palisade parenchyma was aligned regularly and strictly, in three rows, sponge parenchyma cells were containing small intercellular spaces. Many fibrovascular bundles were located in the midrib prominently and shape of the midrib is similar to *E. camaldulensis*. According to our findings after examination of the midrib and the leaf blade cross sections; it was concluded that all leaves samples that are were as *E. globulus* were *E. camaldulensis*.

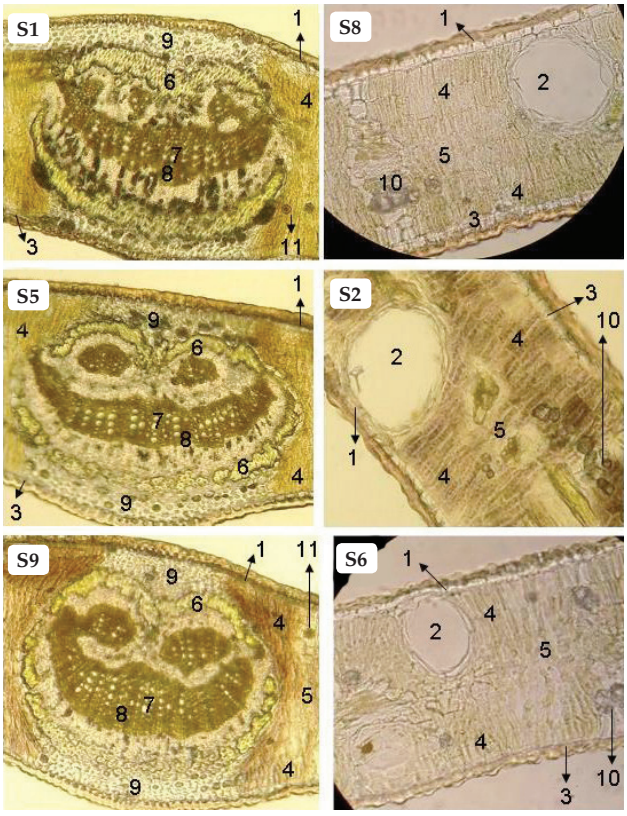

Figure 5. Examples to midrib (10x10) and leaf blade cross-sections (10X40) of the market samples of *Eucalyptus* species. (S:Sample, 1: Upper epidermis, 2: Oil Gland, 3: Lower epidermis, 4: Palisade parenchyma, 5: Sponge parenchyma, 6: Sclerenchima, 7: Xylem, 8: Phloem, 9: Collenchyma, 10: Calcium oxalate crystals, 11: druse)

Essential Oil Content of the Reference Samples: Essential oil of the reference samples collected from Tarsus in November was obtained and oil percentages were found to be 0.86 ml/g in *E. camaldulensis*, 2.71 ml/g in *E. globulus* and 1.07 ml/g in *E. grandis*. These essential oils were used as reference in TLC analysis of three oil samples (VO1, VO2, and VO3).

TLC Analysis of Essential Oils and Leaf Extracts: A strong, dark pink 1,8-cineol spot in both VO1 and VO2 was observed in the chromatogram of essential oil samples (Figure 6). The 1,8-cineol spot in the VO1 was bigger than the spots in all reference essential oils. Thus, addition of 1,8-cineol to the product VO1 was considered. Additionally, an opaque trace was mentioned in VO2 chromatogram that might have been a sign of fixed oil adulteration to the product VO2. The TLC profile of VO3 was extremely different from the other samples and the reference essential oils (EC, EGL, and EGR). Additionally, when toluene was added to dilute VO3, turbidity occurred in the vial. When the odor of VO3 was carefully examined, a minty fresh menthol smell was realized. After a second TLC analysis with VO3 and menthol, the same menthol spots were seen in the chromatogram with same Rf values. No spot belonging to 1,8-cineol and other constituents of *Eucalyptus* species was seen in the chromatogram

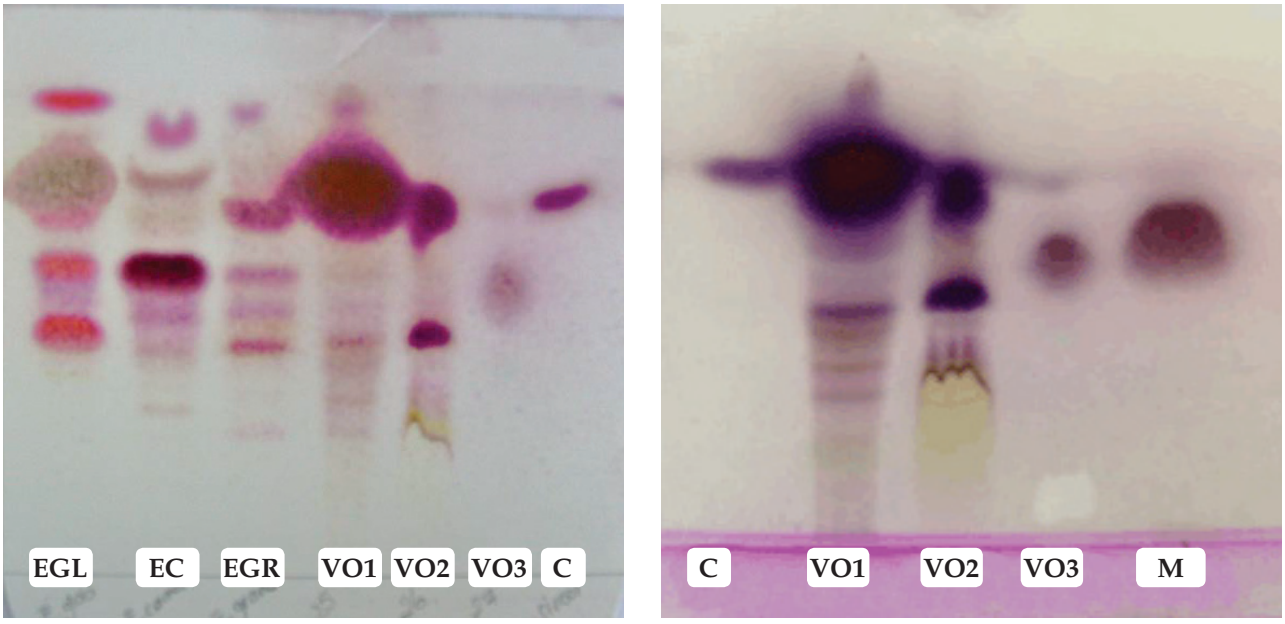

Figure 6. Chromatograms of Essential Oil Samples
(C:1.8-cineol, EC: *E. camaldulensis*, EGL: *E. globulus*, EGR: *E. grandis*, M:Menthol, VO: Volatile oil samples)

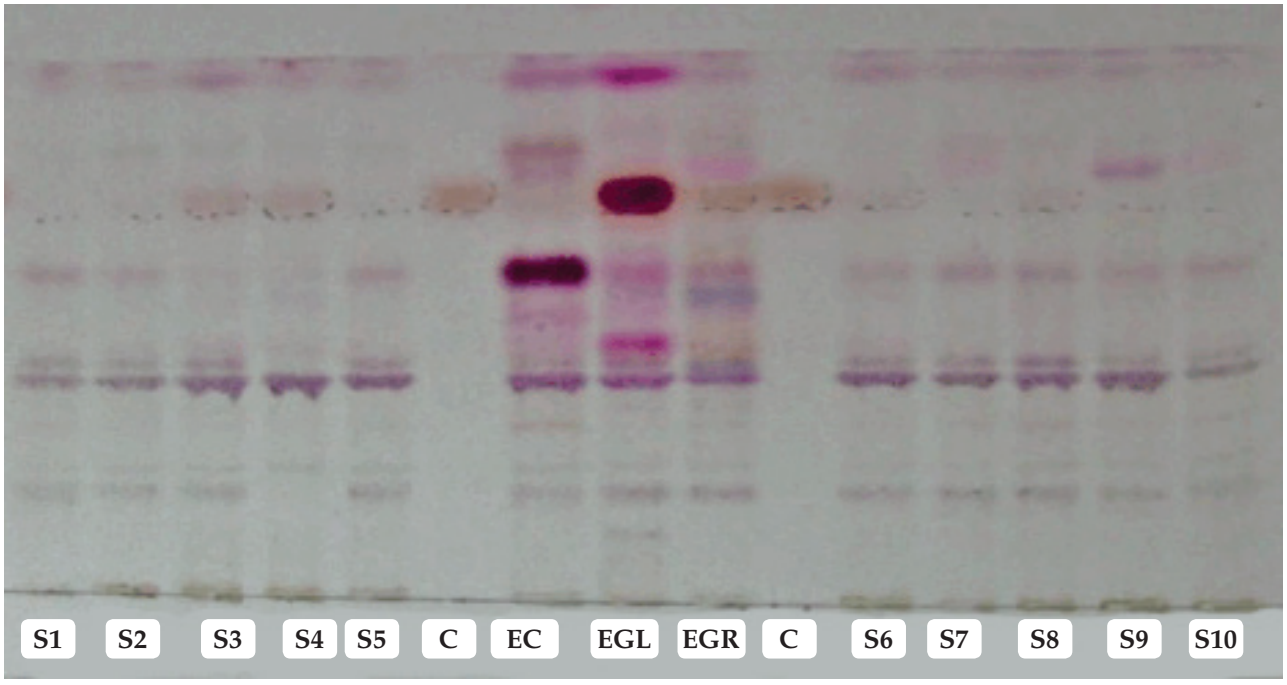

Figure 7. Chromatograms of leaf extracts of the samples
(C:1.8-cineol, EC: *E. camaldulensis,* EGL: *E. globulus,* EGR: *E. grandis,* S:Sample)

of VO3. As a result, three essential oil samples were found to be different from the essential oil of official species *E. globulus* with some adulterations.

In the TLC analysis of the extracts prepared from leaves of market samples and references; 1,8-cineol showed a dark pink zone in the middle after spraying with anisaldehyde and heating (Figure 7). A dark pink spot with same Rf value was seen in all samples that proved the presence of 1,8-cineol in the leaf samples. The darkest and biggest spot was seen in *E. globulus* (EGL). In addition, an intense violet zone of hydrocarbons near the solvent front was seen in all samples and the references. TLC profiles of all market samples were similar to *E. camaldulensis.* None of the market samples contained a similar spot to the pink spot in *E. globulus* and to the blue spot in *E. grandis.* The results proved that market samples included same compounds with *E. camaldulensis* and these samples belonged to *E. camaldulensis.*

CONCLUSION

This study is the first market research on *Eucalyptus* products sold in Turkey. According to the results of morphological, anatomical and chromatographic investigations; all the market samples sold as the officinal species *E. globulus*, were determined as E. *camaldulensis*. Adulterations of chemical substances and fixed oils were detected in essential oil samples. As a result, these oil samples were found to be different from the essential oil of the official *E. globulus* species.

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