

## Taxonomical Properties of Three *Verbascum* L. Species and Their Antioxidant Activities

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The purpose of this investigation was to determine and characterize the antioxidant activity of the 12 extracts of the aerial parts from three *Verbascum* L. species, of which two are endemic to Turkish Flora, utilizing the DPPH free radical-scavenging and  $\beta$ -carotene/linoleic acid assays. The methanol and water extracts of three *Verbascum* species exerted greater antioxidant activity than those of other plant extracts with an IC value of  $130.8 \pm 0.5$ ,  $309.3 \pm 0.5$ ,  $65.4 \pm 0.5$ ,  $235.6 \pm 0.5$ ,  $123.8 \pm 0.5$  and  $132.8 \pm 0.5$   $\mu\text{g/mL}$  for *V. leptocladum*, *V. mucronatum* and *V. davisianum*, respectively. Furthermore, the morphological and anatomical characters of these *Verbascum* species were described comparatively. In two different test system, the most active plant was *V. mucronatum* with  $65.4 \pm 0.5$   $\mu\text{g/mL}$  and 70.4 (%) inhibition rate. Antioxidant activities of BHT were also used for positive controls. In addition, anatomical structures of root, stem and leaves of three *Verbascum* species are given in this study for the first time. According to results, in terms of root, stem and leaf anatomical structures of each *Verbascum* species, *V. leptocladum* differs morphologically and anatomically from the others. These differences can be summarized as follows; in stem narrower sclerenchymatic cells and phloem than those in *V. leptocladum*; in leaf of *V. leptocladum*; having a protrusion below, continuous vascular bundles, less parenchymatic cells in outer and inner epidermis, stellate type hairs and differences in the number of cells in secretion hairs.

**Key Words:** *Verbascum*, Morphology, Anatomy, Antioxidant activities.

### INTRODUCTION

The genus *Verbascum* L. (*Scrophulariaceae*, Common mullein) includes about 360 species<sup>1</sup>. In Turkey it has 233 species in 13 groups and 126 hybrids<sup>2-5</sup>. These species are distributed in Anatolia and Mediterranean Phytogeographical area<sup>2</sup>.

These species are widely used in folkloric medicine due to their antimicrobial and anticarcinogenic properties<sup>6</sup>. The roots, flowers and leaves of *Verbascum* are also used as anodyne, antiseptic, astringent, analgesic and antihistaminic<sup>7</sup>. The leaves

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and flowers of common mullein are used to treat respiratory problems, gastric ulcer and hoarseness. Antiviral, antibacterial, antifungal, antioxidant and antiinflammatory effect have been reported<sup>8,9</sup>. According to previous investigations of *Verbascum* species. Iridoid glycosides, phenylethanoid glycosides, oleanane-type triterpenes, flavonoids are the most common phytochemicals for this genus. To a lesser extent, saponins, alkaloids, steroids and neolignans have also been reported<sup>10-13</sup>.

*Verbascum* genus is one of the biggest genus in a view of the species containing, generally having problems and also known for several problems in diagnosis and taxonomy. The studies related to morphologic and anatomic properties is little<sup>14,15</sup>.

*Verbascum leptocladum* Boiss. & Heldr. and *V. davisianum* Hub.-Mor. are endemic to Anatolia. *V. mucronatum* Lam. is also grows in Anatolia and Crete Islands. Spreading localization of *V. davisianum* and *V. leptocladum* is quite limited in Turkey<sup>2</sup>.

Both species are found as R category in literature and are of the plants, the kind of which became extinct. It is reported that *V. davisianum* is closely similar to *V. bellum* and *V. pycnostachyum*<sup>16</sup>.

In this research, *V. mucronatum* (Group K) and species of *V. leptocladum* (Group L) and *V. davisianum* (Group K) considering in a risk category have been examined with the aim of taxonomic by comparing to the properties of morphologic, morphometric, anatomic and antioxidant. It is aimed that it should contribute to the Flora of Turkey, systematic of species, group and kind by omitting new characters. In present studies, morphological and morphometric properties in relation with species have been evaluated by comparing to the kinds in Flora of Turkey<sup>2</sup>.

The anatomic properties of the species have been studied with the aim of helping the systematic of taxa and have been compared to the studies done long before. So, systematic data gathering has been aimed to determine more natural classifications. In addition to this, ethyl acetate, chloroform, methanol and water extracts have been prepared by Soxhlet apparatus from the air-dried of plant materials (*Verbascum leptocladum*, *Verbascum davisianum*, *Verbascum mucronatum* growing in Turkey) and their antioxidant activities have been tested. No previous phytochemical or biological studies on these species have been reported.

## EXPERIMENTAL

**Plant collection and extraction:** The flowering aerial parts of *V. mucronatum* were collected in Turkey, C3 Antalya, Varsak (36°57'36" N, 30°42'37" E), roadsides, 90 m above the sea level, at the middle of June 2007. The flowering aerial parts of *V. leptocladum* were collected in Turkey, C3 Antalya, Akdeniz University Campus (36°54'00" N, 30°38'23" E), under and clearing *Pinus brutia*, 35 m above the sea level, at the middle of June 2007. The flowering aerial parts of *V. davisianum* were collected in Turkey, C3 Antalya, Feslikan Yaylasi (36°48'54" N, 30°23'30" E), pasture and limestone scree, 1885 m above the sea level, at the middle of July 2007. Voucher specimens of *V. leptocladum*, *V. mucronatum* and *V. davisianum* are deposited in the Herbarium of the Biology Department, Akdeniz University in Antalya, Turkey and

Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir, Turkey (ESSE). For anatomical studies, plant materials have been preserved in 70 % alcohol.

**Morphological:** The materials were identified as *V. leptocladum*, *V. mucronatum* and *V. davisianum* using Flora of Turkey and the East Aegean Islands<sup>2</sup>. Herbarium specimens were used for description of species and detailed morphological drawings. General appearances have been drawn for determination of morphological characters of taxa and added shapes of leaf, bract, bracteol, calyx, corolla and fruit. A Wild M5 A stereo microscope with drawing tube was utilized for morphological drawings.

**Anatomical:** For anatomical studies, samples have been collected from their natural habitats and kept in 70 % alcohol. In the research, root, stem and leaves of mature and flowered plants have been used. Investigations were performed on the cross-sections of the leaf, the flowering stem and root. The anatomical structures of glandular and covering hairs were drawn using Leitz SM-LUX binocular microscope.

In the extraction procedure, the air-dried and finely ground plant materials (the flowering aerial parts of three *Verbascum* species) were extracted in Soxhlet apparatus with ethyl acetate, chloroform, methanol and water at 60 °C. The extracts was then filtered and concentrated *in vacuo* at 45 °C.

#### Antioxidant activity

**DPPH Assay:** The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay (Pharmacia, Uppsala, Sweden) LKB-Novaspec II) uses stable radical diphenylpicrylhydrazyl (DPPH)<sup>17</sup> as a reagent (Sigma-Aldrich). Various concentrations of the extracts (50 µL) in methanol was added to 5 mL of a 0.004 % methanol solution of DPPH. After 0.5 h incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in per cent (I %) was calculated as follows:

$$I \% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test compound) and  $A_{\text{sample}}$  is the absorbance of the test compound. Extract concentration providing 50 % inhibition ( $IC_{50}$ ) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate and butylated hydroxytoluene (BHT) was used as positive control.

**β-Carotene-linoleic acid assay:** In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation<sup>18</sup>.

## RESULTS AND DISCUSSION

**Morphological results:** Morphological and morphometric findings belonging to *Verbascum mucronatum*, *Verbascum davisianum* and *Verbascum leptocladum* have been given in Table-1. In this table, data obtained as a result of the study have

TABLE-1  
MORPHOLOGICAL AND MORPHOMETRICAL CHARACTERISTICS OF *V. mucronatum*, *V. davidianum* AND *V. leptocladium* (Figs. 1-3)

Characters	<i>V. Mucronatum</i>		<i>V. Davidianum</i>		<i>V. Leptocladium</i>	
	Davis	Alan	Davis	Alan	Davis	Alan
Life form	Biennial	Same	Biennial	Same	Perennial	Same
Height of Plant	100-200 cm	95.5-200 cm	30-80 cm	25-100 cm	30-45 cm	30-55 cm
Shape of stem	Very robust terete or obtuse-angled below	Same	Robust, terete	Same	Slender, thin, erect or ascending-erect, terete	Same
Branched	Many-branched	Same	Simple or with few branches below	Same	Usually branched	Same
Surface of stem	Densely white-tomentose or often glabrescent	Same, glandular	Persistent, densely white-pannose, eglandular	Same, glandular	Finely adpressed greyish or whitish floccose-tomentose, glabrescent, eglandular	Same, glandular
Size of basal leaves (cm)	30-65 × 10-25	13-65 × 4-24	5-9 × 2-5	4.5-20 × 1.5-12	2-10 × 0.5-2	3-13 × 0.5-3
Shape of basal leaves	Ovate to oblong-lanceolate	Same	Obovate	Same	Narrowly oblong, ligulate	Elliptic oblong
Base of basal leaves	–	Decurrent	–	Obtuse	–	Acute
Margin of basal leaves	Coarsely crenate or crenate-dentate	Same	Crenulate	Same	Obscurely crenulate-serrate or entire	Obscurely crenulate
Apex of basal leaves	Acutish or acute	Same	Acute-mucronate	Same	Obtus or acutish	Same
Surface of basal leaves, cauline leaves, bracts and bracteols	–	Densely white-tomentose, glandular	–	Persistent, densely white-pannose, glandular	–	Floccose-tomentose, glabrescent, glandular
Size of petiole (cm)	Indistinct or to 8	Same	2-5	2-6	1-4	2-4
Size of cauline leaves (cm)	–	3-30 × 2-15	–	6-19 × 2.5-5	–	1.5-9 × 0.4-1.5
Shape of cauline leaves	–	Ovate, oblong-lanceolate	–	Ovate	–	Linear, oblanceolate

Base of cauline leaves	Broadly decurrent	Same	Decurrent	Same	—	—
Margin of lamina	Crenulate to entire	Same	Subentire	Same	—	Entire
Apex of cauline leaves	Caudate	Same	—	Mucronate	—	Obtus
Number of flowers	3-9	Same	2-7	Same	(1-)2-3	Same
Shape of inflorescence	Broad ovate panicle	Same	Compact cylindrical	Same	Broad panicle	Same
Size of inflorescence (cm)	—	To 40	—	10-21	—	To 30
Size of bracts (cm)	—	0.7-3 × 0.6-2.5	—	1.5-9 × 1-4	—	0.5-1.5 × 1-2
Shape of bracts	Ovate to linear	Same	Triangular to lanceolate	Obovate-lanceolate	Linear-oblong	Linear, triangular
Base of bracts	—	Decurrent	—	Decurrent	—	Rotundate or subcordate
Margin of bracts	—	Crenate	—	Entire	—	Entire
Apex of bracts	Caudate	Same	—	Mucronate, acuminate	Blunt or acutish	Acute-acuminate
Size of bracteoles (mm)	—	2-5 × 1-2	—	3-7 × 1-4	—	3-5 × 0.9-1
Shape of bracteoles	Lanceolate	Same	Lanceolate	Triangular-lanceolate	Linear-oblong	Same
Margin of bracteoles	—	Entire	—	Entire	—	Entire
Apex of bracteoles	—	Subulate	—	Acuminate-subulate	—	Acute
Size of pedicel (mm)	3	2-3	Absent or to 2	Same	To 5	Absent or to 3
Surface of pedicel and calyx	—	Densely floccose tomentose, glandular	—	Persistent, densely white-pannose, glandular	—	Floccose-tomentose, glabrescent, glandular
Size of calyx (mm)	3-5	3-7	8-10	7-11	4-7	6-8
Shape of teeth	Lanceolate	Linear-lanceolate	Same	Same	Linear-oblong	Same
Number of teeth	—	5-7	—	5	—	5
Apex of teeth	Acuminate	Acute to acuminate	Acute	Same	Obtuse	Obtus-acute
Colour of corolla	Yellow	Same	Yellow	Same	Yellow	Same
Diameter of corolla (mm)	20-30	15-31	C. 20	20-25	16-22	18-20

Shape of lobes	—	—	—
Number of lobes	—	—	—
Size of lobes (mm)	—	—	—
Surface of corolla	With pellucid glands, sparsely floccose-tomentose	Pellucid glandular, tomentose	Without pellucid glands, stellate-tomentose
Number of stamen	5	5	5
Size of stamen (mm)	—	—	—
Shape of anthers	Reniforme	Reniforme	Reniforme
Size of anthers (mm)	—	—	—
Size of filaments (mm)	—	—	—
Colour of hairs	Whitish-yellow	Whitish-yellow	Whitish-yellow
Hairs	Wool up to anthers	Wool up to anthers	Wool up to anthers
Size of ovary (mm)	—	—	—
Shape of ovary	—	—	—
Size of style (mm)	—	—	—
Surface of style	—	—	—
Shape of stigma	—	—	—
Shape of capsule	Ovate-globose	Elliptic	Ovate-oblong
Size of capsule (mm)	4-7 × 4-6	7-9 × 4-5	3-5 × 2.5-3.5
Surface of capsule	Tomentose-glabrescent	Densely tomentose, glabrescent	Tomentose
Shape of seed	—	—	—
Size of seed (mm)	—	—	—
Colour of seed	—	—	—
	Rotate	Oblong	Rotate
	5	5	5
	5-12	5-10	5-8
	Same	Same	Same
	1-2	1-2	1-2
	4-6	4-5	4-5
	Same	Same	Same
	Same	Same	Same
	1.5-2 × 1.5-2	2-5 × 2-4	1.5-2 × 1.5-2
	Oblong-cylindrical	Oblong-cylindrical	Oblong-cylindrical
	3.5-5	4-9	3.5-5
	Glabrous	Glabrous	Tomentose
	Capitate	Capitate	Capitate
	Same	Orbiculate	Same
	3-7 × 3-6	7-10 × 4-6	2.5-5 × 2-4
	Same	Same	Same
	Triangular-rotundate	Rotundate	Orbiculate
	0.5-1 × 0.4-0.7	0.9-1.2 × 0.9-1	0.7-1 × 0.5-0.9
	Brown	Brown	Black-brown

been evaluated by comparing to descriptions given in Flora of Turkey<sup>2</sup>. Whereas it is seen that examined samples are generally harmonious with the characters given, some characters have been determined that they are not the same in Flora of Turkey<sup>2</sup>. In addition, in Table-1, new characters, important for the systematic of species and genus, added to the characters mentioned in Flora of Turkey, have been designated. The forms of important characters in view of designation of this species have been shown in the Figs 1-3.

**Anatomical results:** The sections taken from root and stem leaves of *V. mucronatum*, *V. davisianum* and *V. leptocladum* are examined and the anatomic features belonging to plants are determined, compared and showed in Figs. 4-13.



Fig. 1. *Verbascum mucronatum*





Fig. 2. *Verbascum davisianum*



Fig. 3. *Verbascum leptocladum*. (A) Habit. (a) Basal leaves. (b) Cauline leaves. (c) Bract. (d) Bracteole. (e) Calyx and pistile. (f) Corolla. (g) Stamen. (h) Capsule. (i) Seed

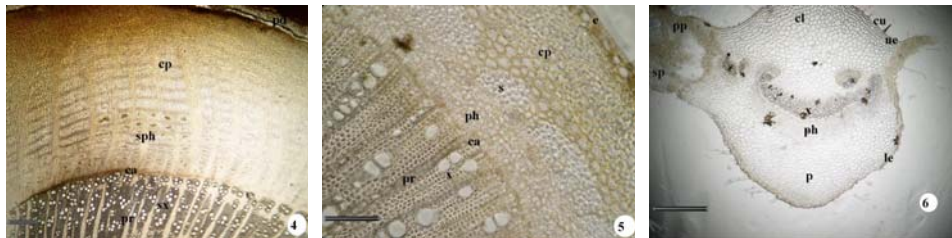


Fig. 4-6. *V. mucronatum* 4. Cross-section of root. 5. Cross-section of stem. 6. Cross-section of leaf in LM, respectively



Fig. 7-9. *V. davisianum* 7. Cross-section of root. 8. Cross-section of stem. 9. Cross-section of leaf in LM, respectively



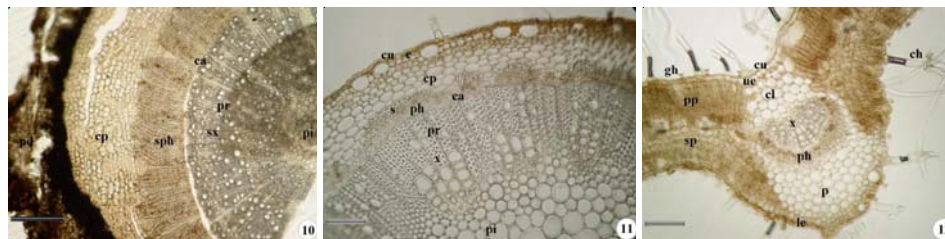


Fig. 10-12. *V. leptocladum* 10. Cross-section of root. 11. Cross-section of stem. 12. Cross-section of leaf in LM, respectively; cu-cuticle; e-epidermis; cl-collenchyma; cp-cortex parenchyma; pd-peridermis; pr-pith ray; pi-pith; s-scleranchyma; ph-phloem; x-xylem; sph-secondary phloem; sx-secondary xylem; ch-covering hair; ue-upper epidermis; le-lower epidermis; gh-glandular hair; p-parenchyma; pp-palisade parenchyma; sp-spongy parenchyma. Bar = 30  $\mu$

**Root:** Root in all the species is formed by periderm on the outside and felloderm where 4-5 radial row are broken down and felloderm with 2-3 row tissues. Outer felloderm cells are broken up or tissue residues belonging to primer cortex is time to time crushed are found. Secondary floem formed of elliptical-shapeless, round shaped, irregular-arranged and 4-6 row cells under the periderm is taken part. Cambium is uncertain. Seconder xylem part cover a large area and consist of tracheal elements with big and small sizes in a sclerenchymatic tiissue. Pith branches are 2-3 row cells. Pith region covering a narrow area is parenchymatic in *V. mucronatum* and *V. leptocladum* and is sclerenchymatic in *V. davisianum* (Figs. 4, 7, 10).

**Stem:** When the cross sections are taken on the stems of three *Verbascum* species, secondary development have been observed. Epidermis is formed by single row, thick membrane elliptic or round cells. Upper or lower walls are thick but lateral sides are thin. Its upper surface is covered with cuticle (Figs. 5, 8, 11). Covering hair and glandular trichomes are observed. Covering hairs of *V. mucronatum* are candelabriform and multicellular. Glandular trichomes are 5 types; head 1 stalk 2 celled, head 2 stalk 2 celled, head 3 stalk 2 celled, head 1 stalk 3 celled and head 1 stalk 1 pellucid celled (Fig. 13A). They are candelabriform and multicellular in *V. davisianum*. Its glandular trichomes three types; head 1 stalk 2 celled, head 2 stalk 2 celled, head 2 stalk 3 celled (Fig. 13B), stellate and multicellular in *V. leptocladum*. Its glandular trichomes are seven types; head 1 stalk 1 celled, head 2 stalk 1 celled, head 1 stalk 2 celled, head 2 stalk 2 celled, head 3 stalk 2, head 1 stalk 3 celled, head 2 stalk 3 celled (Fig. 13C). Parenchymatic cortex in 8 or 10 rows is found in all species under the epidermis. Collenchyma cells under the epidermis in primer cortex are seen, on the other hand parenchyma cells including oval-shaped chloroplast inside the epidermis are found. Druse crystals are observed in parenchymatic cells. Endodermis consisting of flattened cells can hardly be distinguished from the cortex parenchyma. It has been seen that there are sclerenchyma bunches such as big, small and discontinuous, 1 or 2 rows in seconder phloem of *V. leptocladum*, but 4-6 rows on the other types. Phloem is much narrower in *V. leptocladum*, 3-4 rows,

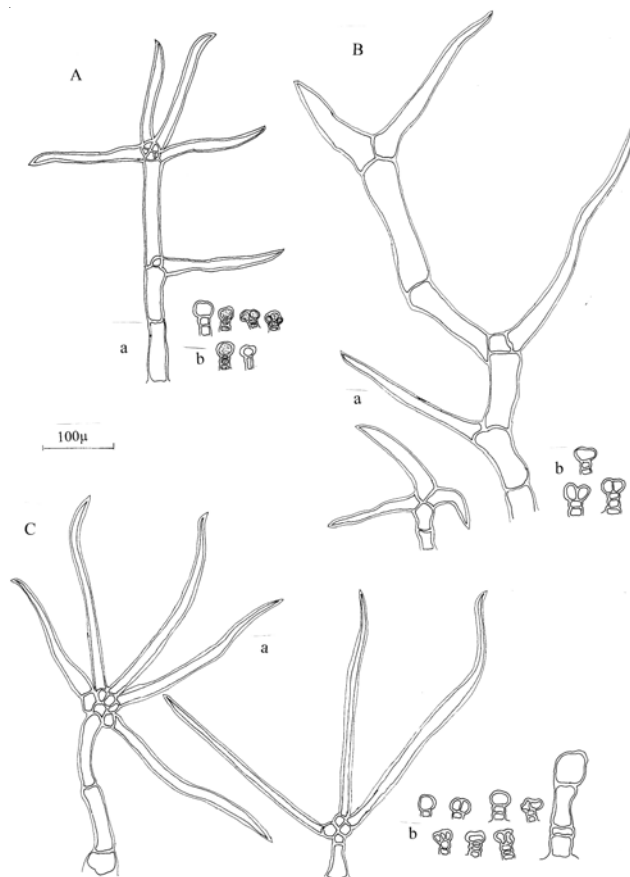


Fig. 13. Trichomes of stem and leaf. (A) *V. mucronatum*, (B) *V. davisianum*  
(C) *V. leptocladum*, (a) eglandular trichomes (b) glandular trichomes

but it is much wider in *V. mucronatum* and *V. davisianum* and 3-8 rows, but it is a circle shape consisting of flattened, shapeless or oval cells. Cambium is uncertain. Branches in all types of secondary xylem are especially getting narrow towards primary xylem. Sclerenchymatic cells in this part formed from tracheae and tracheids in all types have been created regular rows in radial direction. Pith branches are in the form of large polygon or round shaped and parenchymatic cells where its walls are lignified. Druse crystals are clearly found in these cells (Figs. 5, 8, 11).

**Leaf:** In cross-section of main and intervascular tissues outer and inner layers of *V. mucronatum* and *V. davisianum*, but only inner layer of *V. leptocladum* are clearly protrusive. Epidermis includes single flattened row, rectangular, round or oval shaped cells. Outer epiderm cells are bigger than inner epiderm cells, being covered with a thin cuticle layer and curly over. Outer membranes are thicker than inner and longitudinal membranes, but it has been observed that inner membranes of epiderm cells in the middle vein area became thicker. Covering hairs and glandular

TABLE-2  
ANATOMICAL DIFFERENCES OF THE SPECIES

	<i>V. mucronatum</i>	<i>V. davisianum</i>	<i>V. leptocladum</i>
Root	Pith parenchymatic	Pith sclerenchymatic	Pith parenchymatic
Stem	Sclerenchyma	4-6 celled	4-6 celled
	Phloem	Wide, 3-8 celled	Wide, 3-8 celled
			Narrow, 3-4 celled
	Above and below of leaf	Protrusion	Protrusion
	Vascular bundles	Discontinuous	Discontinuous
Leaf	Outer epidermis	10-12 celled	10-12 celled
	Parenchymatic cells		
	Inner epidermis	4-20 celled	4-20 celled
	Parenchymatic cells		5-10 celled
Eglandular hairs in the stem and leaf	Candelabriform multicellular	Candelabriform multicellular	Stellate multicellular
Glandular hairs in the stem and leaf	Head 1 stalk 2 celled; head 2 stalk 2 celled; head 3 stalk 2 celled; head 1 stalk 3 celled; head 2 stalk 3 celled; pellucid glands	Head 1 stalk 2 celled; head 2 stalk 2-3 celled; pellucid glands	Head 1 stalk 1-2-3 celled; head 2 stalk 1-2-3 celled; head 3 stalk 2 celled

trichomes are the same as the stem and are seen in both epidermis. Covering hairs are candelabriform and multicellular in *V. mucronatum*. Glandular trichomes are 5 types; (Fig. 13A). Covering hairs of *V. davisianum* are candelabriform and multicellular. Glandular trichomes are 3 types; (Fig. 13B). Covering hairs are stellate and multicellular in *V. leptocladum*. Glandular trichomes are 7 types; (Fig. 13C). Stoma (amphistomatic) are found in both surfaces of the leaf are much denser on the lower surface. They are on much more upper limit of epidermis cells in cross-section (hygromorphic stoma) Mesophyll in all types has been arranged as a two or three-row under the outer epiderm. It has been formed with plenty of chloroplasted palisade parenchyma and three or five rows sponge parenchyma which is under it (bifacial leaf). Vascular bundles are collateral. They are well developed and interrupted in *V. leptocladum*, but interrupted crescent shaped in other types. Xylem is placed in outer epiderm, but phloem in inner epiderm. Tracheal elements in xylem are arranged like radial are found parenchymatic cells with thin walls. Phloem is placed under the xylem. Clear parenchymatic cells are placed in a 1 or 2 rows under the outer epiderm, three or five rows in *V. leptocladum* after collenchyma and 10 or 12 rows in other two types. Parenchymatic tissue has been found in *V. mucronatum* and *V. davisianum* with 4-20 rows under the phloem till inner epiderm, but much narrower in *V. leptocladum* with 5-10 rows. Thick lateral veins in both sides of middle vein are arranged till edge of palm and middle vein has made a deep outgrowth. Lateral veins in view of anatomic are the same structure as middle veins, but vascular are much more reduced (Figs. 6, 9, 12).

Extracts obtained by Soxhlet extraction, from the aerial parts of the plants studied, were individually assessed for their possible antioxidant activities by employing two complementary tests: DPPH-free radical scavenging and  $\beta$ -carotene/linoleic acid assays. Free radical scavenging capacities of corresponding extracts were measured by DPPH assay and inhibition percentages of the linoleic acid oxidation by the extracts and their results are shown in Table-3.

TABLE-3  
FREE RADICAL-SCAVENGING CAPACITIES OF THE EXTRACTS MEASURED BY  
DPPH ASSAY AND INHIBITION PERCENTAGES OF THE LINOLEIC ACID  
OXIDATION BY THE EXTRACTS

Extracts	DPPH assay IC <sub>50</sub> ± 0.5 (µg/mL)	$\beta$ -Carotene assay inhibition* (%)
<i>Verbascum leptocladum</i>		
MeOH	130.8	32.5
H <sub>2</sub> O	309.3	26.4
EtOAc	Non-active	33.6
CHCl <sub>3</sub>	Non-active	31.8
<i>Verbascum mucronatum</i>		
MeOH	65.4	70.4
H <sub>2</sub> O	235.6	22.4
EtOAc	Not dissolved	Not dissolved
CHCl <sub>3</sub>	Not dissolved	Not dissolved
<i>Verbascum davisianum</i>		
MeOH	123.8	33.2
H <sub>2</sub> O	132.8	30.1
EtOAc	Non-active	35.6
CHCl <sub>3</sub>	Non-active	48.7
BHT (Positive control)	19.8	100.0

\*2 mg/mL concentration for extracts.

As shown in Table-3, free radical-scavenging activity of methanolic and water extracts of *Verbascum* species were superior to other plant extracts studied. In the  $\beta$ -carotene/linoleic acid system (Table-3) oxidation of linoleic acid was effectively inhibited by methanolic extract of *V. mucronatum* (70.4 %), followed by chloroform extract of *V. davisianum* (48.7 %) and ethyl acetate extract of *V. davisianum* (35.6 %). Water extract of *V. mucronatum* exhibited the weakest antioxidant activity (22.4 %). Furthermore, *V. mucronatum* showed the highest antioxidant activity in both assay. Antioxidant activity of BHT was also determined as positive control in parallel experiments.

Number of studies related to genus *Verbascum* are comparatively less<sup>14</sup>. Therefore, the species belonging to the genus are not many in view of comparing with each other. In this studies, the morphological, anatomical and antioxidant properties of *V. mucronatum*, *V. davisianum* and *V. leptocladum* species have been determined.

*V. mucronatum* and *V. davisianum*, the East Mediterranean element, have been found in group K and *V. leptocladum* is in group L in the Flora of Turkey<sup>2</sup>. Hair structure of other species of bracte and calyx, which are found in this group, is separated from being decurrent of cauline leaves, calyx length, indumentum structure and margin form diversity of basal leaves<sup>2</sup>. Morphological properties are given in Table-1 determined in relation to these species have generally indicated conformity with Flora of Turkey. But, in present findings, the basal leaves of length-width and pedicel height are lower than the values mentioned in Flora of Turkey. While calyx in Flora of Turkey is 5 mm, it has been measured as 7 mm as a result of present observations. The low limit of corolla's diameter has also been found much lower in present findings. While the plant length in *V. davisianum* has been indicated as 30-80 cm in Flora of Turkey in puts, it has been measured as 25-100 cm in present findings. The length of basal leaf (length-width), different from lower and upper limits of 5-9 × 2-5 cm. Whereas bracts shape is triangular to lanceolate and bracteol shape is lanceolate in Flora of Turkey, bracte shape is obovate-lanceolate and bracteol shape is triangular-lanceolate in present studies. Capsule has been observed as orbiculate not elliptic, which is different from Flora of Turkey<sup>2</sup>. Whereas *V. leptocladum* is measured as 45 cm of the upper limit of plant length in the Flora of Turkey<sup>2</sup>, it has been measured as 55 cm as a result of present findings. In addition, while basal leaf length is 2-10 × 0.5-2 cm and its margins are obscurely crenulate-serrate or entire in the Flora of Turkey, it is 5-15 × 0.5-2 cm and obscurely crenulate in present findings. Its leaf shape is narrowly oblong and ligulate in Flora of Turkey, it has been found as elliptic oblong in present results. Lamina type and its apex of bracte have been mentioned as linear-oblong and blunt/acutish, it has been found as triangular and its apex is acute-acuminate in present observations. In addition to this, while pedicel length has been measured till 5 mm in Flora of Turkey, it has been found as absent or to 3 mm in present findings. The top of calyx teeth is almost obtuse in Flora of Turkey, while it has been determined as obtuse-acute in present observations. Furthermore, the stem hairs in the description belonging to the species have been mentioned as eglandular, while stem, basal leaf, stem leaf, bracts and bracteols are glandular hair in present observations. These diversities are connected with the example number examined and ecological reasons. Moreover, some other morphological characters not mentioned in the description of the Flora of Turkey have been searched for the purpose of contributing to being defined of taxonomic relations among species and the systematic of species. These properties are the base shape of basal leaf, the lower and upper part of hair, the lamina form of stem's leaf, length-width, the lower and upper surface hair state; the inflorescence length, the bract length-width, the base of bract, the margin, the lower and upper surface hair state; the bracteol length-width, the base of bracteol, its margin, the top, the lower and upper state of its hair, the pedicel position, the hair position, the number of calyx teeth, its hair state, the form of corolla lobes, the number of them, the length, the stamen, anther, the filament length, ovary length, its form, the hair state, the style length, its hair state, the

stigma form, the seed form, its length-width and the colour. The differences among species have been given as comparison in Table-1. According to this, *V. mucronatum*, *V. davisianum* are biennials but *V. leptocladum* perennial. The biggest distinction among these three species is the plant length, the branch shape and whether the basal leaves of stem are decurrent, the calyx length, the indumentum structure and pedicel lengths. With these species of characters, it has been observed that *V. leptocladum* is different from the other two species. *Verbascum* growing naturally in Turkey taken part in *Bothosperma* section of Murbeck<sup>19</sup> have been divided into 13 groups in Flora of Turkey. Whereas *V. mucronatum* and *V. davisianum* are taken part in Group K of these 13 group and *V. leptocladum* in Group L, *V. natolicum* (A), *V. euphraticum* (F), *V. diversifolium* and *V. birandianum* (G), *V. melitenense* (Group H) studied species early. The most important characters separating these groups and species reported from each other are lamina margin and end of basal leaf, the branching and blooming shapes, or whether bractes are found or not, the numbers of stamen and flower, the flower size, the hairy state, the hair colour of filament, the hair state of capsule. In addition, the feature separating Group K and Group L from each other is the length according to calyx of pedicel length.

Its anatomic structure belonging to its root, stem and leaves of *V. mucronatum*, *V. davisianum* and *V. leptocladum* samples has been given in this study for the first time. The anatomical differences comparatively among species as a result of the researches have been given in Table-2. According to these results, as well as every one of three *Verbascum* species has distinctive properties in view of morphological characters and examined root, stem and leaf anatomies, on account of anatomical properties too, *V. leptocladum* has shown different properties from other two species. These differences can be put in order that sclerenchyma bundles in the stem and floem in *V. leptocladum* are much narrower; only the lower surface is protruding again in *V. leptocladum* in view of leaf anatomical properties; vascular bundles are uninterrupted; upper and lower epiderm's parenchyma cells are much less and glandular and covering hairs are different. The anatomical properties belonging to three species have generally shown coincidence with findings of Metcalfe and Chalk signified in the genus<sup>15</sup>.

The importance of these characters has increased once more on account of *V. leptocladum* taking part in the most dangerous category, *V. davisianum* endemic species, being under the less risk category in literature<sup>16</sup>. Consequently, it is obligation that these species encountering with little by little disappearing danger are protected in Botanical gardens. It is important that the completed research introduces the species in view of systematical situation because there isn't a research done with the aim of taxonomical about these species of the genus. The importance of species has redounded on account of especially both species under the disappearing danger, having narrow spread and being endemic species.

The results of antioxidant activities obtained from extracts and findings determined for positive control BHT have been shown in Table-3.



As a result of present experiment, the methanolic and water extracts of *V. mucronatum*, *V. davisianum* and *V. leptocladum* have shown high activity. Even, *V. mucronatum* is the most active plant from the the other two *Verbascum* species.

### ACKNOWLEDGEMENTS

The authors are grateful to Akdeniz University, Scientific Research Project Unit for financial support to collect the plant samples.

### REFERENCES

1. V.H. Heywood, Flowering Plants of the World, Oxford University Press, New York, pp. 27-35 (1993).
2. A. Huber-Morath, in ed.: P.H. Davis, *Verbascum* L., Flora of Turkey and the East Aegean Islands Edinburgh University Press, Vol. 6, pp. 461-603, ISBN 0-85224-3367 (1978).
3. P.H. Davis, R.R. Mill and K. Tan, in eds.: P.H. Davis, R.R. Mill and K. Tan, *Verbascum* L., Flora of Turkey and the East Aegean Islands (Suppl.), Edinburgh University Press, Vol. 10, pp. 191-193 (1988).
4. T. Ekim, in eds.: A. Güner, N. Özhatay, T. Ekim, K.H.C. Baser, *Verbascum* L., Flora of Turkey and the East Aegean Islands (Suppl.), Edinburgh University Press, Vol. 11, p. 193. ISBN-13: 978-0748614097 (2000).
5. A.F. Karaveliogullari, A. Duran and E. Hamzaoglu, *Annales Botanici Fennici*, **41**, 227 (2004).
6. M. Sengül, H. Ögütçü, A. Adigüzel, F. Sahin, A.A. Kara, I. Karaman and M. Güllüce, *Turk. J. Bot.*, **29**, 105 (2005).
7. F. Senatore, D. Rigano, C. Formisano, A. Grassia, A. Basile and S. Sorbo, *Fitoterapia*, **78**, 244 (2007).
8. N. Aligiannis, S. Mitaku, E. Tsitsa-Tsardis, C. Harvala, I. Tsaknis, S. Lalas and S. Haroutounian, *J. Agric. Food Chem.*, **51**, 7308 (2003).
9. I. Gürbüz, A.M. Özkan, E. Yesilada and O. Kutsal, *J. Ethnopharmacol.*, **101**, 313 (2005).
10. H. Abougazar, E. Bedir, I.A. Khan, I. Çalis and A.E. Wiedemannioside, *Planta Medica*, **69**, 814 (2003).
11. I. Tatli, Z.S. Akdemir, E. Bedir and I.A. Khan, *Turk. J. Chem.*, **28**, 111 (2004).
12. K. Drandarov and M. Hesse, *Tetrahedron Lett.*, **43**, 5025 (2002).
13. Z.S. Akdemir, I. Tatli, E. Bedir and I.A. Khan, *Turk. J. Chem.*, **28**, 621 (2004).
14. T. Cakir and E. Bagci, *Firat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, **18**, 445 (2006).
15. C.R. Metcalfe and L. Chalk, *Anatomy of the Dicotyledons*, 1, Oxford University Press, London, edn. 2 (1979).
16. IUCN, IUCN Red List Categories, Version 3.1. Prepared by the IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK. ISBN-13: 978-2831706337 (2001).
17. M. Cuendet, K. Hostettmann and O. Potterat, *Helv. Chim. Acta*, **80**, 1144 (1997).
18. C. Barriere, D. Centeno, A. Lebert, S. Leroy-Setrin, J.L. Berdague and R. Talon, *FEMS Microbiol. Lett.*, **201**, 181 (2001).
19. S. Murbeck, *Monographie der Gattung Celsia*. Lunds Universitat Arssk., Vol. 22, pp. 1-241, (1925).