Pharmaceutical Botanical and Phytochemical Investigation of *Chelidonium majus* L. (Papaveraceae) Growing in Eskişehir

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ABSTRACT

In this study, the morpho-anatomical features and volatile components of *Chelidonium majus* L were investigated. *C. majus* L., the only species of the *Chelidonium* L. genus in our country, is known locally as 'Temre otu' or 'Kırlangıçotu'. This species is recorded for the first time in Flora of Turkey records for square B3. Morphological structures of flower, leaf and stem were examined and measurements were taken. Cross sections from the root and body of the plant, cross and superficial sections from the leaf were taken and anatomically examined. The essential oil obtained from the above-ground parts of the plant was analyzed by GC and GC/MS. The main compounds of the essential oil of C. majus were phytol (22.4%), methyl linolenate (13.2%) and nonacosan (6.5%).

Keywords: Anatomy, *Chelidonium majus*, essential oil, GC-GC/MS, morphology

Eskişehir'de Yetişen *Chelidonium majus* L. (Papaveraceae) Bitkisinin Farmasötik Botanik ve Fitokimyasal Olarak İncelenmesi

ÖZ

Bu çalışmada, *Chelidonium majus* L. bitkisinin morfo-anatomik özellikleri ve uçucu bileşenleri araştırılmıştır. *Chelidonium* L. cinsinin ülkemizdeki tek türü olan *C. majus* yöresel olarak 'Temre otu' veya 'Kırlangıçotu' isimleriyle bilinmektedir. Bu tür Flora of Turkey kayıtlarına göre B3 karesi için ilk kez kaydedilmiştir. Bitkinin çiçek, yaprak ve gövdesinin morfolojik yapıları incelenerek ölçümleri alınmıştır. Bitkinin kök ve gövdesinden enine kesitler, yapraklarından ise enine ve yüzeysel kesitler alınarak anatomik incelemeleri yapılmıştır. Bitkinin toprak üstü kısımlarından elde edilen uçucu yağın GC ve GC/MS analizleri yapılmıştır. *C. majus* bitkisinin uçucu yağının başlıca bileşikleri fitol (%22,4), metil linolenat (%13,2) ve nonakosan (%6,5) olarak bulunmuştur.

Anahtar Kelimeler: Anatomi, *Chelidonium majus*, GC, GC/MS, morfoloji, uçucu yağ

INTRODUCTION

The Papaveraceae family spans Europe and Asia, comprising roughly 28 genera and approximately 250 species globally. Within Turkey, this family includes five genera and fifty species. They are perennial or annual, rarely dwarf shrub-like herbaceous plants. They are woody at the base and bear transparent or coloured milk tubes [1]. The genus *Chelidonium* L., which is named as 'Kırlangıçotu' in Turkish, is distributed with a single species in our country [2].
Chelidonium majus L., which has a very widespread use in the world and in our country in terms of its use among the people, was first encountered in Ebers papyri 3500 years ago, and its effectiveness against cancer was mentioned in a herbal treatment book published in 1536. *C. majus* is traditionally employed for treating various conditions, including eczema [3], infections of viral, bacterial, and fungal origins, as well as providing anti-inflammatory [4], pain-relieving, anti-diarrheal [5], anti-tumor effects [6] and antifungal [7], [8] fresh latex is used externally against warts and fresh leaf juice is used against eye diseases of infectious origin, blood-letting, eczema, blood purifier [9]. *C. majus* has shown significant improvement in anorexia, vomiting, diarrhea, weight loss and biochemical values in cats and dogs [10]. It is also used for gall bladder diseases, liver, diarrhoea and loss of appetite. Its ingredients have weak painkillers and antispasmodic properties. Oral consumption of *C. majus* in humans has been associated with the risk of causing significant hepatic toxicity [11], [12]. Its roots, when chewed in the mouth, relieve toothache. The decoction boiled on fire has an eye-strengthening effect [13]. When there is blindness in swallow chicks, the mother brings these plants to the

chicks and rubs them and makes them see [14]. It has been determined that it has a potentially effective role in the treatment of ovarian cancer. [12]. In addition to its antiviral effects against the COVID-19 pandemic, it is thought that it may be effective against the COVID-19 virus by strengthening liver function. [15]. Again, researchers have revealed that phytocomponents of *C. majus* can be used as antidiabetic and antiobesity [16].

C. majus, a member of the Papaveraceae family, is a medicinal plant that grows in the Mediterranean Europe and Asia phytogeographic region [15]. The above-ground parts of the plant collected during the flowering period are used to obtain Herba Chelidonii [17]. This plant has been used medicinally for many years for the eczema, treatment of skin cancer, infection, some venereal diseases and the prevention of kidney stones. In addition, chelidonine and stilopin have been shown to have a cytotoxic effect on cancer cells such as prostate, breast, lung, liver and colon [6]. There are biological activity studies of essential oil and its extracts [6], [18]–[22].

The aim of this study is to determine the morphological characteristics of *C. majus*, the only species of the genus *Chelidonium* in our country, to present them in comparison with the Flora of Turkey, and to clarify its anatomical characteristics. Another aim of the study is to reveal the volatile oil composition of this plant, which is of ethnobotanical importance.

MATERIALS AND METHODS

Plant Materials

The research material was collected B3: Eskişehir in Dağküplü Village of Sarıcakaya District (Figure 1). The plant specimens were identified with the help of volume 1 of Davis' "Flora of Turkey and The East Aegean Islands" [1]. Some of the plant samples were converted into herbarium specimens by assigning herbarium numbers and are preserved in ESSE (Anadolu University Faculty of Pharmacy Herbarium) (ESSE NO: 15535) (Figure 2). Plant identification was made by Prof. Dr. Sevim KÜÇÜK and Dr. Sıraç TOPDEMİR. Some of the fresh specimens were placed in a jar with 70% alcohol for morpho-anatomical studies and to preserve freshness.

Figure 1. *C. majus* L. (Photo by: S. Topdemir and Ü. Çelik)

Figure 2. *C. majus* L. herbarium specimen (ESSE NO: 15535)

Morphological Studies

The identification of the *C. majus* specimens was carried out using the Flora of Turkey [15, 16]. The morphological characteristics of the taxon were examined in detail and compared with the characters given in Flora. Detailed measurements were taken from various parts of the plant to support identification and comparison.

Anatomical Studies

Anatomical investigations were conducted on the root, stem, and leaves of the plant. Transverse sections were prepared from the root, stem, and both the upper and lower surfaces of the leaf. These sections were stained using Sartur Reagent and subsequently mounted in glycerin-gelatin for microscopic examination.

Essential Oil Extraction

Essential oil of the aerial parts of the plant material (100g) was obtained by Clevenger Apparatus. Fresh sample was used, volumetric moisture content was determined in parallel with water distillation and essential oil yield was calculated from the dry plant (Yield 0.03 %). The essential oil analysed by GC and GC/MS system [23].

Gas Chromatography and Gas Chromatography - Mass Spectrometry Analysis (GC-GC/MS)

GC-MS Conditions:

The essential oil was analyzed using a capillary GC/MS system (Agilent GC-MSD, Agilent Technologies Inc., Santa Clara, CA, USA). Separation was performed on an HP-Innowax fused silica capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness; Hewlett-Packard, USA). Helium was used as the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was initially held at 60°C for 10 minutes, then increased to 220°C at a rate of 4°C/min and held at 220°C for 10 minutes. Subsequently, the temperature was increased to

240°C at 4°C/min. The injector temperature was maintained at 250°C. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV with a mass scan range of *m/z* 35–450 [24].

GC Conditions:

Gas chromatography was conducted using an Agilent 6890N GC system equipped with a flame ionization detector (FID) set at 300°C. To ensure consistency in elution order with GC-MS, a duplicate analysis was performed using the same HP-Innowax column and identical oven temperature programming. Simultaneous automatic injections were applied to both systems. The relative percentage compositions of the oil components were calculated based on the peak areas obtained from the FID chromatograms, without applying correction factors [24].

Identification of Compounds

The identification of essential oil constituents was performed by comparing their mass spectra with those in the Baser Essential Oil Constituents Library, Wiley GC/MS Library, Adams Library, and MassFinder Library, and further confirmed by comparison of retention indices. A homologous series of n-alkanes was used as reference standards for the calculation of relative retention indices (RRI). The relative percentages of the separated compounds were calculated based on the FID chromatograms.

RESULTS

Morphological Observations

The morphological characteristics of *C. majus* are given in Table 1. In this table, the morphological characteristics are compared with those in Flora. In addition to the characteristics mentioned in Flora, new morphological characteristics that may be important for the identification and description of *C. majus* have been determined in Table 1.

Papaveraceae

Herbs, often woody at the base, with colored or transparent sap. Leaves are usually alternate, occasionally opposite, exstipulate, and often deeply divided. Sepals 2(-3), free, usually caducous. Petals 4(6), free or slightly united at the base. The corolla can be actinomorphic or zygomorphic. Stamens numerous, or ranging from 6 to 4. Ovary superior, syncarpous, composed of 2-20 carpels. The fruit may be a capsule, nut, lomentum, or siliquiform. Seeds range from one to numerous [1].

Chelidonium

According to our findings, the plant is perennial, 29-32 cm in height, has 5 leaflets, upper leaflets 28-49 mm, lower leaflets 10-38 mm, leaf color is green. Leaf type unite, leaf top obtuse, leaf base cuneate, leaf edge lobed, leaf arrangement imparipinnate, flower condition racemus, corolla color yellow, corolla 4-lobed. Runway 4-10 mm, stamens 4-5 mm, anther color yellow, fruit 7-37 mm [1].

Per Davis' records, the species is documented across grid squares A1 through A9, with this study providing the first recorded occurrence in grid square B3 [1].

The comparison of morphological research findings of *C. majus* species with Flora of Turkey (Table 1) [1].

Table 1. Comparison of morphometric characteristics of *C. majus* L. with the Flora of Turkey

Characters	**Flora of Turkey**	**Our findings**
Plant	Perennial	Perennial
Plant height	90 cm	29 – 32 cm
Leaflet number	5 - 7	5
Upper leaflet	Unspecified	28 – 49 mm
Lower leaflet	Unspecified	10 – 38 mm
Leaf colour	Unspecified	Green
Leaf type	Unspecified	Unite
Leaf top	Unspecified	Obtus
Leaf base	Unspecified	Cuneate
Leaf edge	Unspecified	Lobed
Leaf arrangement	Unspecified	Imparipinnate
Flower condition	Unspecified	Rasemus
Corolla colour	Unspecified	Yellow
Number of corolla lobes	Unspecified	4
Runway	Unspecified	4 – 10 mm
Stamen	Unspecified	4 – 5 mm
Anther colour	Unspecified	Yellow
Fruit	Unspecified	7 – 37 mm

Anatomical Features

Root

The protective tissue layer, the periderm, consists of 3-5 rows of actinomorphs with disrupted fellems and 4-6 rows of felloderma. The outermost cork cells are damaged (fragmented) or have partially crushed tissue remnants of the primary cortex. The secondary phloem, which is arranged in 6-8 rows below the periderm, consists of irregularly arranged, ring-shaped, oval, shapeless cells. Secondary xylem covers a large area and tracheal elements form a sclerenchymatic basic tissue in large and small diameters. The pith arms consist of 1-2 rows. The pith region covers a narrow area and is not parenchymatic (Figure 3).

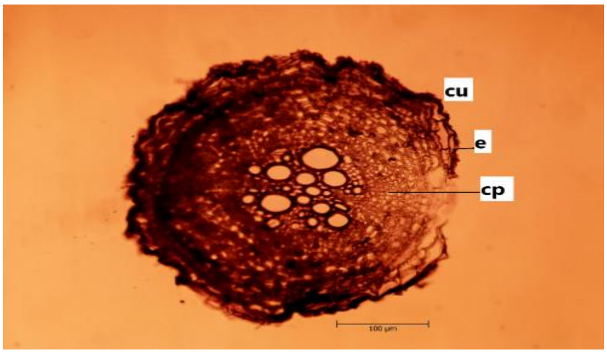

Figure 3. *C. majus*. Root cross-section (x10), (e: epidermis, cp: cortex parenchyma, cu: cuticula)

Stem

While the upper and lower walls of the epidermis are thick, the lateral walls are thin, the epidermis consists of round or oval 2-row cells, and the cells are thick-walled. The epidermis is also covered with a thin cuticle. Cover hairs are simple hairs with 5-10 cells. The parenchymatic cortex has 6-10 rows and is located just below the epidermis. In the inner parts of the body there are parenchyma cells (oval shaped) containing chloroplasts, while in the primary cortex, collenchyma cells are located below the epidermis. Drus crystals were observed in the parenchyma cells. The conduction bundles form regular radial rows of 10-11 bundles in the parenchymatic tissue with phloem elements facing outwards and xylem elements facing inwards. Phloem is composed of amorphous (flattened) or oval cells, primary xylem is composed of tracheae and tracheids. The cambium is prominent. The pith branches are arranged in 1-2 rows and are composed of parenchymatic cells. Lysigen spaces (latex) were observed in these cells. Drus crystals are absent (Figure 4).

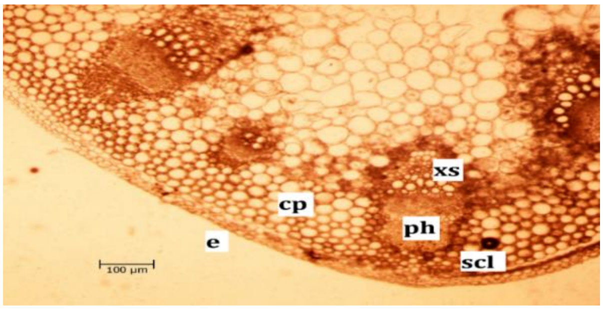

Figure 4. *C. majus*. Stem cross-section (x40), e: epidermis, ph: phloem, cp: cortex parenchyma, xs: xylem, scl: sclerencima

Leaf

The upper epidermis has 1-2 rows of round shaped cells and 1-2 rows of palisate parenchyma underneath. In addition, the sponge parenchyma under the lower epidermis has 2-3 rows of oval or round cells. The conduction bundle is wrapped in a bundle sheath with the phloem facing the lower epidermis and the xylem facing the upper epidermis. Phloem and xylem structures are in normal condition and are observed in the middle part. Upper epidermis cells in the upper transverse section and lower epidermis cells in the lower transverse section are smooth-walled. Stomatal cells were observed on the lower surface (Figure 5, Figure 6).

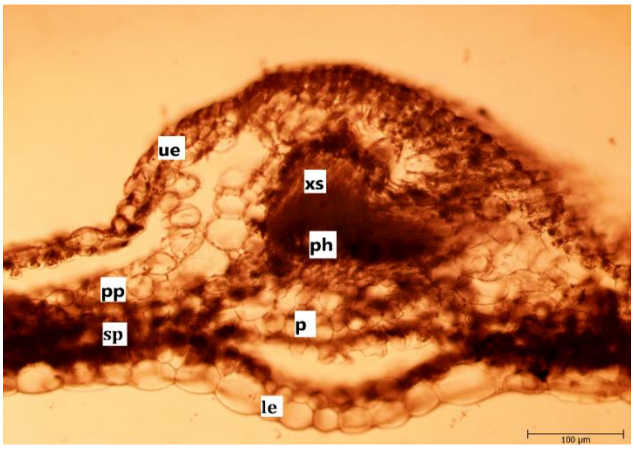

Figure 5. *C. majus* (ESSE 15535). Leaf cross-section (x40), le: lower epidermis, ph: phloem, xs: xylem, cu: cuticula, p: parenchyma, pp: palisate parenchyma, sp: sponge parenchyma, ue: upper epidermis.

Bottom Surface Cross-Section

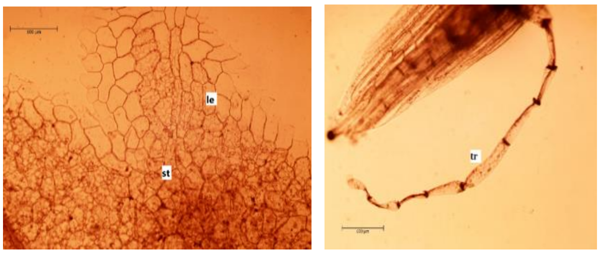

Figure 6. *C. majus*. Leaf lower surface cross-section (x10-x40), le: lower epidermis, st: stoma, tr: trichome

Upper Surface Cross Section

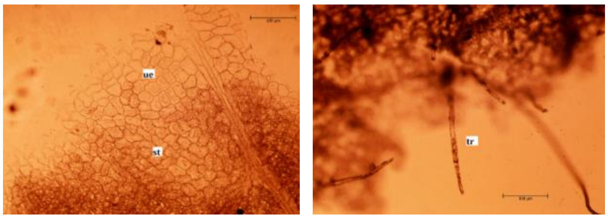

Figure 7. *C. majus*. Leaf upper surface cross-section (x10-x40), ue: upper epidermis, st: stoma, tr: trichome

Essential Oil

Table 2. Essential oil composition of *C. majus* L.

RRI	Compound	%	IM
1203	Limonene	0.9	t_R, MS
1553	Linalool	0.2	t_R, MS
1554	*trans*-Chrysanthenyl acetate	2.0	MS
1612	β-Caryophyllene	0.7	t_R, MS
1600	Hexadecane	0.5	t_R, MS
1668	(Z)-β-Farnesene Beta	3.1	MS
1700	Heptadecane	1.0	t_R, MS
1722	Dodecanal	0.4	t_R, MS
1726	Germacrene D	1.4	MS
1758	(*E,E*)-α-Farnesene Alfa	1.4	MS
1800	Octadecane	2.1	t_R, MS
1900	Nonadecane	2.5	t_R, MS
1925	Ionol	0.6	MS
1932	Neophytadiene isomer I	0.8	MS
1992	Neophytadiene	1.3	MS
2000	Eicosane	0.3	t_R, MS
2041	Pentadecanal	2.4	MS
2100	Heneicosane	3.8	t_R, MS
2131	Hexahydrofarnesyl acetone	2.0	t_R, MS
2200	Docosane	1.3	t_R, MS
2226	Methyl hexadecanoate	2.9	MS
2240	1-Methyl ethyl hexadecanoate	0.5	MS
2241	Heptadecanal	1.0	MS
2296	Isophytol	1.2	MS
2300	Tricosane	3.3	t_R, MS
2384	1-Hexadecanol	1.3	MS
2400	Tetracosane	0.9	t_R, MS
2464	Nonadecanal	1.7	MS
2500	Pentacosane	4.1	t_R, MS
2509	Methyl linoleate	2.4	t_R, MS
2583	Methyl linolenate	13.2	MS
2607	1-Octadecanol	1.7	MS
2600	Hexacosane	1.1	t_R, MS
2622	Phytol	22.4	MS
2700	Heptacosane	3.4	t_R, MS
2900	Nonacosane	6.5	t_R, MS
	Monoterpene hydrocarbons	0.9	
	Oxygenated monoterpenes	0.2	
	Sesquiterpenes hydrocarbons	5.2	
	Oxygenated sesquiterpenes		
	Diterpenes	25.7	
	Others	64.3	
	Total %	96.3	

CONCLUSIONS and DISCUSSION

Although there are very few morpho-anatomical studies [4] on *C. majus*, the only species of the genus *Chelidonium* L. in our country, known locally as 'Temre otu' or 'Kırlangıçotu', the specimens collected from Eskişehir region were studied by us for the first time.

As a result, morphological and anatomical properties of *C. majus* is parallel in the literature, except for some differences in size or number. Some character measurements made in the *C. majus* species differed from the values in Flora [1].

Morphological characters such as plant height and number of leaflets differed from Davis' Flora of Turkey. While the plant height was 90 cm according to Davis, it was 29-32 cm according to our findings. While the number of leaflets was between 5-7 according to Davis, it was 5 in our findings. Other characters are not described in Flora of Turkey and the differences are given in Table 1 [1].

The morphological characters that we found in our study findings and not mentioned in the Flora are added to the literature.

Lower leaflets, leaf color, leaf type, leaf top, leaf base, leaf margin, leaf arrangement, flower condition, corolla color, number of corolla lobes, pistil, stamen, anther color, fruit diameter are morphological characters added.

The anatomical structure of the species (root-stem-leaf) was revealed for the in this study. Zare et al. (2021) reported that the periderm is generally detached from the cortex and that the cortex consists of 20–35 layers; these findings are consistent with our observations. However, in our study, the periderm consists of 3–5 rows of actinomorphic cells and 4–6 rows of felloderm, providing more detailed structural information. Additionally, secondary xylem in our samples occupies a large area, and the tracheary elements form a sclerenchymatic tissue, whereas Zare et al. (2021). provided less detail on the conducting tissues. In both studies, the endodermis is indistinct, which appears to be a common anatomical feature. The diarch primary structure observed in our study indicates a young root anatomy and aligns with Zare et al. findings [4]. The stem anatomy in both studies is largely consistent. Zare et al. (2021) reported a collenchymatous outer region and a multilayered parenchymatous cortex, which is also clearly observed in our study. In our samples, the vascular bundles are arranged in a single ring with 12–14 bundles, xylem elements are V-shaped, and the cambium is well-developed; these details were not provided in Zare et al. (2021)'s work. Both studies note the presence of latex throughout the stem and the location of laticiferous tubes near the phloem. Calcium oxalate crystals in parenchymatous cells were observed more clearly in our study. Both studies demonstrate that the leaves are bifacial (dorsiventral) with a palisade layer located beneath the upper epidermis. Zare et al. (2021) reported that the leaves are hypostomatic with stomata confined to the lower epidermis; our study confirms this observation [4]. Our study provides more detailed micromorphometric data, including epidermal cell size, stomatal index, stomatal type (anomocytic), and the thickness of palisade and spongy parenchyma layers. Vascular arrangement in both studies shows xylem adaxial and phloem abaxial positioning. Additionally, collenchyma layers and calcium oxalate crystals were observed in the main vein region in our study, which were not described in Zare et al. (2021)'s work [4].

Overall**,** the fundamental anatomical features in both studies are in agreement; however, our study provides more detailed data, particularly regarding vascular structure, cell morphology, latex distribution, and crystal formation.

The above-ground parts of *C. majus* were collected from Sarıcakaya district of Eskişehir in April 2019 and essential oil was obtained from the above-ground parts by hydrodistillation. The essential oil yield obtained from the above-ground parts of *C. majus* was 0.03%. GC-GC/MS systems were used for essential oil determination and 36 compounds representing 96.3% of the essential oil were characterized. The identified compounds are shown in Table 2.

Phytol (22.4%), methyl linolenate (13.2%), and nonacosane (6.5%) were identified as the predominant components in the essential oil derived from *C. majus* (Table 2). Phytol is used in the fragrance industry, cosmetics, shampoos, cleaning materials [20]. Phytol appears to possess a wide range of biological activities, including antioxidant, analgesic/antinociceptive, anti-inflammatory, and cytotoxic/antitumor potential in both in vivo and in vitro models. However, dose/formulation is critical in terms of efficacy and safety; structural derivative studies enhance this potential [25]–[27]. In the study, the cytotoxicity of phytol, a diterpene alcohol, was evaluated in vitro using the MTT assay on seven human tumor cell lines and one normal cell line. The tested compound induced a concentration-dependent cytotoxic response in all cell lines and was shown to be most effective against breast adenocarcinoma MCF-7 and least effective against prostate adenocarcinoma PC-3 cells (IC50 8.79 ± 0.41 mM and 77.85 ± 1.93 mM, respectively). IC50 values against the other five tumors (HeLa, HT-29, A-549, Hs294T, and MDA-MB-231) were reported to range from 15.51 to 69.67 mM [28]. In another study, the HPTLC method was used to determine the amount of bioactive compounds. The GC-HRMS method identified phytol as the active compound that induces ROS-mediated apoptosis in *Schizosaccharomyces pombe* [29].

Methyl linoleate has a role as a plant metabolite. It is commonly used in food, agriculture, lubricants, coatings, metalworking fluids and biofuels [21]. Methyl linoleate is seen as a promising component, particularly in the context of skin/pigmentation (melanogenesis) control; it has biological potential due to its antioxidant or fatty acid content. However, its pharmacological range and depth of effect (e.g., systemic anti-inflammatory, anticancer, antimicrobial, etc.) have been less studied [21], [30]. In a study conducted on mice, it was reported that both methyl oleate and methyl linoleate (400 dose (mg/kg) survival days: 29.5 ± 5.1, lifespan increase: 145.8%) are toxic to tumor cells, but their cytotoxic potential is lower than that of oleic acid and linoleic acid [31]. Nanocosan is a major component in the waxes of the leaf cuticle of many open-seeded plants and some closed-seeded plants and is responsible for the tubular leaf microstructure [22].

Although there are some essential oil studies on *C. majus* in the literature, no study on this species has been found in Turkey.

Compared the chromatographic results of the products of 4 different cosmetic companies in their study and found that the main component of herbal cosmetic products of all companies was phytol (22.64%, 15.21%, 27.74%, 13.8%). The amount of phytol main component in this study is consistent with our results [32].

In another study, different parts of *Chelidonium majus* plant were examined as root, stem, flower and leaf. L-Phenylalanine in root, Tartaric acid and Galactose in stem, L-Alanine, Oxalic acid, Propanol in flower, Lactic acid, L-Valine, Isoleucine, Oxamic acid in leaf were determined as the main components [33]. Deng et al. added two new triterpenes to the literature [34].

Studies have shown that *C. majus* has antibacterial, antiviral, antifungal, spasmolytic, cytotoxic, hepatoprotective activity [17] and is also used in digestive system, hemorrhoids, jaundice, liver, eye and skin diseases [4] Therefore, we think that our study will make important contributions.

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