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A Preliminary Phytochemical, Pharmacognostic and Physicochemical Evaluation of Endemic *Thymus spathulifolius* (Lamiaceae)

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Abstract

Aim of study: The members of the genus *Thymus* are widely used in Turkey as herbal teas, spices and salads for different purpose and the herbs of *Thymus spathulifolius* are reported to have medicinal value. Therefore, in this study various pharmacognostic parameters, physicochemical properties and anatomical characters of *Thymus spathulifolius* Hausskn. & Velen., is a medicinal plant and endemic to Turkey, were investigated.

Area of study: T. spathulifolius was dried and stored according to herbarium techniques in the Herbarium of Pharmacy Faculty, Trakya University, collected from Sivas, Turkey.

Material and methods: The total ash, moisture content, and physicochemical tests on the different extracts obtained from the endemic *T. spathulifolius* aerial parts collected from Sivas province were determined according to the World Health Organization (WHO) standards for crude drugs.

Main results: The moisture content and total ash amount of the drug obtained from this plant is in accordance with the WHO standards. Phytochemical screening for secondary metabolites showed the presence of carbohydrates, antraquinone glycosides, steroids, flavonoids and coumarins, while other phytochemicals such as alkaloids and saponins were absent. Besides, the cross-sections of root, stem and leaf were examined and detailed. The total ash value was found as 7.36 ± 0.21% and acid insoluble ash was 0.14 ± 0.72%.

Highlights: The present study provided important information for correct authentication and standardization of this plant material.

Keywords: *Thymus spathulifolius*, Phytochemical, Microscopic, Physicochemical Parameters

Endemik *Thymus spatulifolius* (Lamiaceae) 'un Ön Fitokimyasal, Farmakognostik ve Fizikokimyasal Açıdan Değerlendirilmesi

Öz

Çalışmanın amacı: Thymus cinsi üyeleri Turkiye'de bitkisel çay, baharat ve salata olarak farklı amaçlarla yaygın kullanılmaktadır. Bu çalışmada, Türkiye'ye endemik olan ve tıbbi değeri olan *Thymus spathulifolius* Hausskn. & Velen bitkisi farmakognozik parametreler, fizikokimyasal ve anatomik özellikleri açısından incelenmiştir.

Çalışma alanı: Thymus spathulifolius Türkiye'nin Sivas ilinden toplanmış ve herbaryum tekniklerine göre kurutularak Trakya Üniversitesi Eczacılık Fakültesi Herbaryumunda saklanmıştır.

Materyal ve yöntem: Sivas bölgesinden toplanan endemik *T. spathulifolius* türünün toprak üstü ksıımlarından total kül ve nem tayini ve hazırlanan farklı ekstreler üzerinde fizikokimyasal testler droglar için olan WHO standartlarına göre yapılmıştır.

Temel sonuçlar: Bu bitkiden elde edilen drogun nem miktarı ve total kül miktarı Dünya Sağlık Örgütü (DSÖ) standartlarına uygundur. Sekonder metabolitlerden fitokimyasal tarama sonucu karbonhidratlar, antrakinon glikozitleri, steroitler, flavonoitler ve kumarin içerdiği, alkaloit ve saponin gibi fitokimyasallarca yoksun olduğu tespit edilmiştir. Ayrıca kök, gövde ve yaprak enine kesitleri anatomik olarak incelenmiş ve detayları ortaya çıkarılmıştır. Total kül miktarı %7.36 ± 0.21, asitte çözünmeyen kül miktarı ise %0.14 ± 0.72 olarak bulunmuştur.

Araştırma vurguları: Bu çalışma *T. spathulifolius* türünün doğru tanınması ve standardizayonu için önemli bilgiler ortaya koymuş ve yakın türlerle ilgili çalışmalara kaynak oluşturmuştur.

Anahtar Kelimeler: *Thymus spathulifolius*, Fitokimyasal, Mikroskobik, Fizikokimyasal Parametreler

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Introduction

Lamiaceae (Labiatae) family are spread all over the world and includes more than 236 genera and 7173 species (Kubitzki & Kadereit, 2012). This family is economically valuable as a source of commercially important medicinal and aromatic plants such as marjoram, lavender, mint, thyme, rosemary, basil and thyme, and is a rich source of essential oils for the aroma and fragrance industry (Wagstaff et al., 1998). Some members of the family are grown as ornamental plants and used in medicament and traditional medicines in Anatolia, Europe and China (Baytop, 1999; Cui et al., 2003; Rudy, 2004).

The *Thymus* L. is an important genus of the Lamiaceae family and consists of nearly 215 species of herbaceous perennials and subshrubs. The gene center of these plants is known as the Mediterranean region. (Nickavar et al., 2005; Agili, 2014). *Thymus* is represented by 42 taxa, 18 of them are endemic to Anatolia (Güner et al., 2012). The *Thymus* species are commonly used as herbal tea, cold & flu treatment, nausea, stomachache, carminative, antiseptic, an antioxidant material and natural substance for food preservations (Sargın et al., 2015; Paksoy et al., 2016). In addition, some researchers found that the essential oils of some *Thymus* taxa have antibacterial and antifungal properties (Karaman et al., 2001; Celen et al., 2012; Sharafzadeh & Bahmani, 2014; Selvi et al., 2022). The medicinal features of *Thymus* species come substantially from its essential oils that extracted by steam distillation from flowers and leaves.

Thymus spathulifolius Hausskn. & Velen is known as the "kaşık kekiği" in local language (Güner et al., 2012), is a perennial plant and dwarf shrub up to 10 cm high, forming small dense cushions and its aerial shoots are much branched. *T. spathulifolius* is an endemic for Central Anatolia. It distributes in Sivas province and grows near forests at gypseous steppe slopes with about 1500 m altitude (Davis et al., 1982). According to IUCN threatened category, *T. spathulifolius* is under EN (Endangered) (Ekim et al., 2000), increases importance of the current study.

Despite the medicinal greatness of this plant, information on the pharmacognostic parameters for identification of this species are unavailable. Any morphological and anatomical study in detail, except the main morphological knowledge of *T. spathulifolius* in "Flora of Turkey" is not known in the literature (Davis et al., 1982). The purpose of this study is to explore pharmacognostic, physicochemical evaluation, phytochemical screening. Besides, detailed anatomical description of root, stem and leaves of *T. spathulifolius* by using light microscopy.

Material and Methods

Plant samples collected from Sivas province of Turkey were dried on shade place. Fresh plant materials were fixed and conserved in 70% ethyl alcohol until used. Handmade cross-sections were taken from roots, stems and leaves with a razor blade Preparations of plant samples were stained in 1% Alcian blue (Sigma) and 1% Safranin O (Sigma), in a ratio 3/2 (Davis & Barnett, 1997). Sections were kept in dye for about 5 minutes. Semi-permanent slides were grouped using glycerin – gelatine (Jensen, 1962). Vegetative parts of specimens were made using Olympus BX21 light microscopy. Photographs of the sections were taken using Olympus BX51 light microscopy coupled with Olympus DP70 digital camera.

For determining physico-chemical parameters of the powdered drug such as foreign matter percentage, loss on drying, total ash, water soluble and acid insoluble ash, the standard methods in WHO guidelines on quality control methods for medicinal plant material were used (WHO, 1998). For extractive value, coarse powder of plant material (10 g) was extracted separately with 100 mL each of hexane, chloroform, ethyl acetate, methanol, and water for 24h by maceration method and after filtration the extract was concentrated under low pressure to dryness. The remaining extract was stored at 4-8ºC until use. The finely powdered samples were subjected to fluorescence analysis both as it is and after treating with different solvents and reagents against normal and UV lamps with short (254 nm) and long wavelength (366 nm).

Preliminary phytochemical screening of the herbal extracts in different solvents was

undertaken according to the standard methods to detect for the presence or absence of the major phytochemicals such as: alkaloid, amino acid, carbohydrate, glycoside, tannin, saponin, steroid, terpenoid and flavonoid (Trease & Evans, 2004).

Results and Discussion

Overview on Extinction Threat

T. spathulifolius is local endemic and known only two localities that Erzincan and Sivas provinces of Turkey (Davis et al., 1982). In the literature survey, floristic studies made in the years after 1982 could not detect any records from other localities. Within the scope of this study, samples were taken from the population of the Flora of Turkey (Davis et al., 1982) in the province of Sivas and the population status was examined. Unfortunately, we are concerned about the future of the population due to factors such as the expansion of agricultural lands and soil losses due to erosion and we consider that necessary precautions should be taken. In addition, we estimate that the endangerment category of *T. spathulifolius* is likely to be raised to CR (Critically Endangered) in future studies, as a result of a detailed evaluation of the populations in Sivas and Erzincan provinces.

Microscopic Evaluation of The Plant

Root: In cross section, the outermost epidermis is single-layered, isodiametric, and fragmented. The periderm layer covers a large area under the epidermis and its cells are crushed or fragmented. This layer is consisting of phellem (4-5 layered), phellogen (7-8 layered) and phelloderm (2-3 layered). Phelloderm cells are parenchymatic, large and irregular shaped. Parenchymatous phloem is surrounded by sclerenchymatic fibers. Cambium cells are not distinct between phloem and xylem. Secondary xylem occupy a large area with ray parenchyma cell. Xylem rays are 1-2 layered and parenchymatic. Vessel elements cover a large area and vessels are homogenously distributed. Primary xylem elements are located in the center of the root (Fig.1 A, B, C).

Stem: Cross-section of stem is angular shaped. The epidermis consists of isodiametric cells, with thickened external walls and being covered by a thick cuticle. Collenchyma is present in corners. Eglandular and glandular trichomes are seen on the whole surface of epidermis. 1-2 celled eglandular hairs and glandular hairs (capitate and peltate hairs) are seen. Cortex is composed of collenchymatous and parenchymatous cells. Collenchyma tissue consists of irregular cells located just below the epidermis and has 6-7 layers at the corners and 1-2 layers between the corners. Underneath the collenchyma, there is 3-4 layered and crushed parenchymatic tissue. These cells broken and as a result of this disintegration, large gaps occur. The endoderm, forms the innermost border of the cortex, is usually a distinct ring formed of single layered cells. These cells are large, rectangular and sometimes are squashed. Pericycle and cambium are not observed. There is 3-4 layered cork tissue below endodermis. Phloem is a ring formed of 7-8 layered of squashed cells. The pith is surrounded by a ring of xylem and the trachea is circular or oval shaped and the tracheids are polygonal. Medullary rays are one layered and numerous. The pith consists of polygonal or circular parenchymatous cells. These cells after 4-5 rows dried out and disintegrated resulting in a gap in the center of the stem (Figure 1 D, E, F).

Leaves: Adaxial epidermis is similar to abaxial epidermis. These layers consist of a single layer of cells ranging from rectangular to cubic in shape and are covered with a thin cuticle. However, the surface of both epidermis is similar as it is covered with glandular and eglandular trichomes. These trichomes were observed as 1-4 cell glandular and capitate and peltate type glandular trichomes, abundantly dispersed on both surfaces. Capitate glandular hairs are more abundant than others and walls of hairs are stiff and lignified. Stomata epistomatic and occurs on the surfaces of both epidermis. Mesophyll is isobilateral (eqvifacial) and occurs multiseriate palisade tissue. 1-2 seriate spongy parenchyma located around the vascular bundles (Figure 2B). Sclerenchymatic tissue surrounded by bundle sheath in mid-vein, well developed than vascular bundles. Vascular bundles are occurred in a narrow area. (Figure 2 A-F).

Physicochemical Study

The pharmacognostic evaluation of some physico-chemical parameters are useful in building standards for an herbal crude drugs. These parameters are guiding us to detecting of drug adulteration or impropriety. Among the parameters, the ash value gives us an information about impurities and inorganic compounds in plant material. Physicochemical parameters of aerial parts of *T. spathulifolius* were studied as described earlier in the methodology section and the obtained results were presented in Table 1. The total ash value for the plant material was found to be 7.36%, which indicate the presence of silicacious substances coming from soil during collection.

Table 1. Physicochemical analysis of aerial parts of *T. spathulifolius*

No.	Parameters		Values (w/w)
1.	Ash values	Total ash value	7.36 ± 0.21%
		Acid insoluble ash	0.14 ± 0.72%
2.	Extractive values	In n-hexane	2.50 ± 0.3%
		In Chloroform	3.73 ± 0.25%
		In Ethylacetate	6.51 ± 0.1%
		In Methanol	9.17 ± 0.4%
		In water	12.15 ± 0.9%
3.	Sweling index (mL)		Nil
4.	Loss on drying		8.89 ± 0.23%

Different medicinal plants have a distinct therapeutic benefit due to the presence of varied elements such as pectin or hemicelluloses, mucilage, and gum, which results in differing swelling qualities of various herbal materials (Pandiyan & Ilango, 2022). The swelling index of *T. spathulifolius* aerial parts was zero, as given in Table 1, which means that the constituents responsible for the swelling properties are absent. The moisture content of the crude drug affects its stability due to in suitable temperature moisture can lead to activation of enzymes and proliferation of living organisms. The higher of the moisture content in crude drug, the higher chance of microbiological growth or activation of enzymes and it will be the lower stability of the drug. The powdered *T. spathulifolius* plant material showed 8.89% value of loss on drying. Therefore, the powdered material should be kept with care in dried form to increase stability by avoiding microbiological reproduction. Extraction value also an important quality control parameter for herbal drugs. In this work, various solvents such as hexane, chloroform, ethyl acetate, methanol and water are used to determine the extractive value. The results showed the polarity of the solvent increases, the yield of the extract increases.

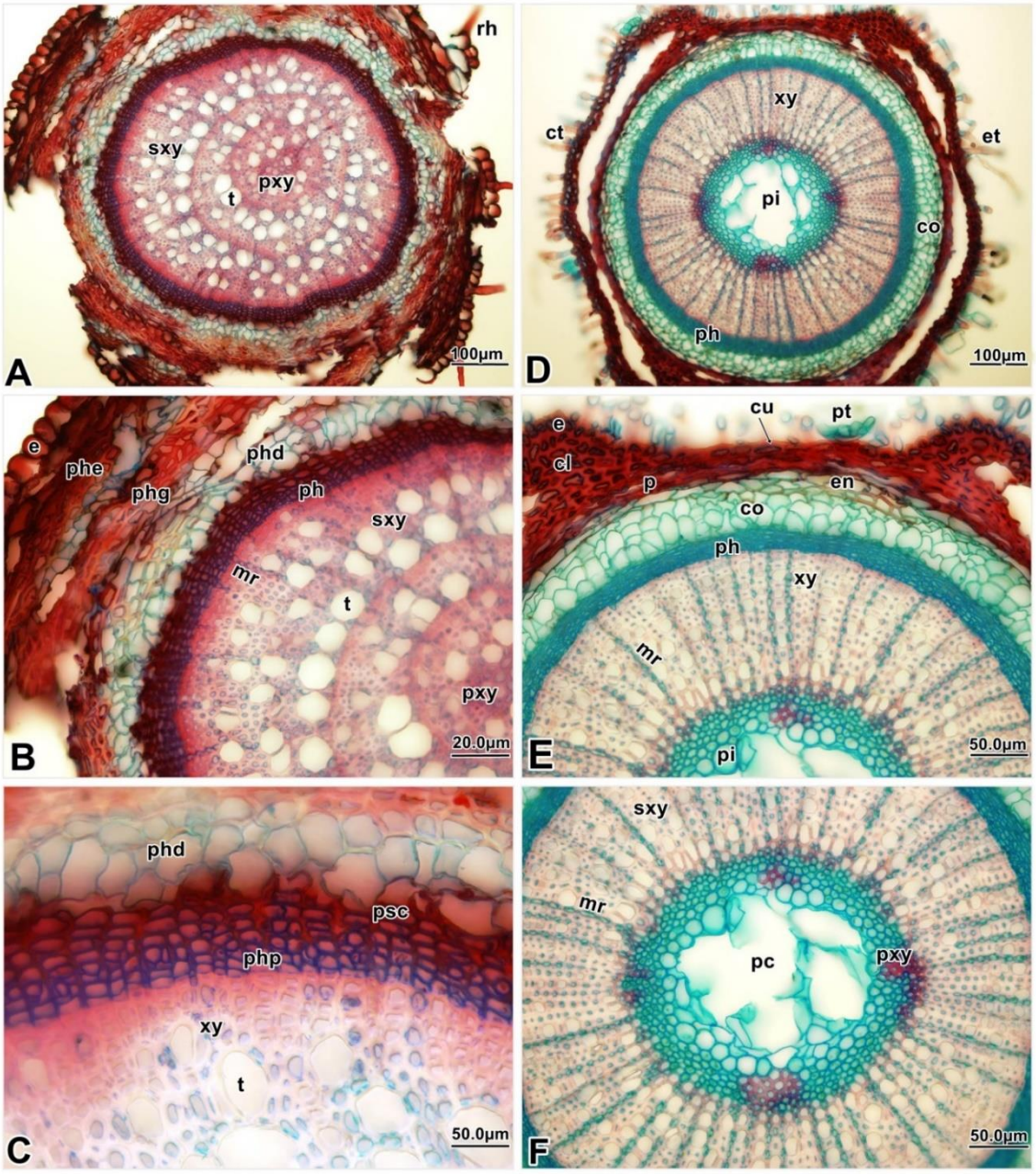

Figure 1. Photographs of a microscopic of the root (A,B,C) and stem (D,E,F) cross-sections of *T. spathulifolius*.

ct: capitate trichome, cl: collenchyma, co: cork, cu: cuticle, e: epidermis, en: endodermis, et: eglandular trichome, mr: medullary rays, p: parenchyma, pc: pith cavity, ph: phloem, pi: pith, php: phloem parenchyma, psc: phloem sclerenchyma, phd: phelloderm, phg: phellogen (cork cambium), phe: phellem, pi: pith, pt: peltate trichome, pxy: primary xylem, rh: root hair, sxy: secondary xylem, t: trachea, xy: xylem.

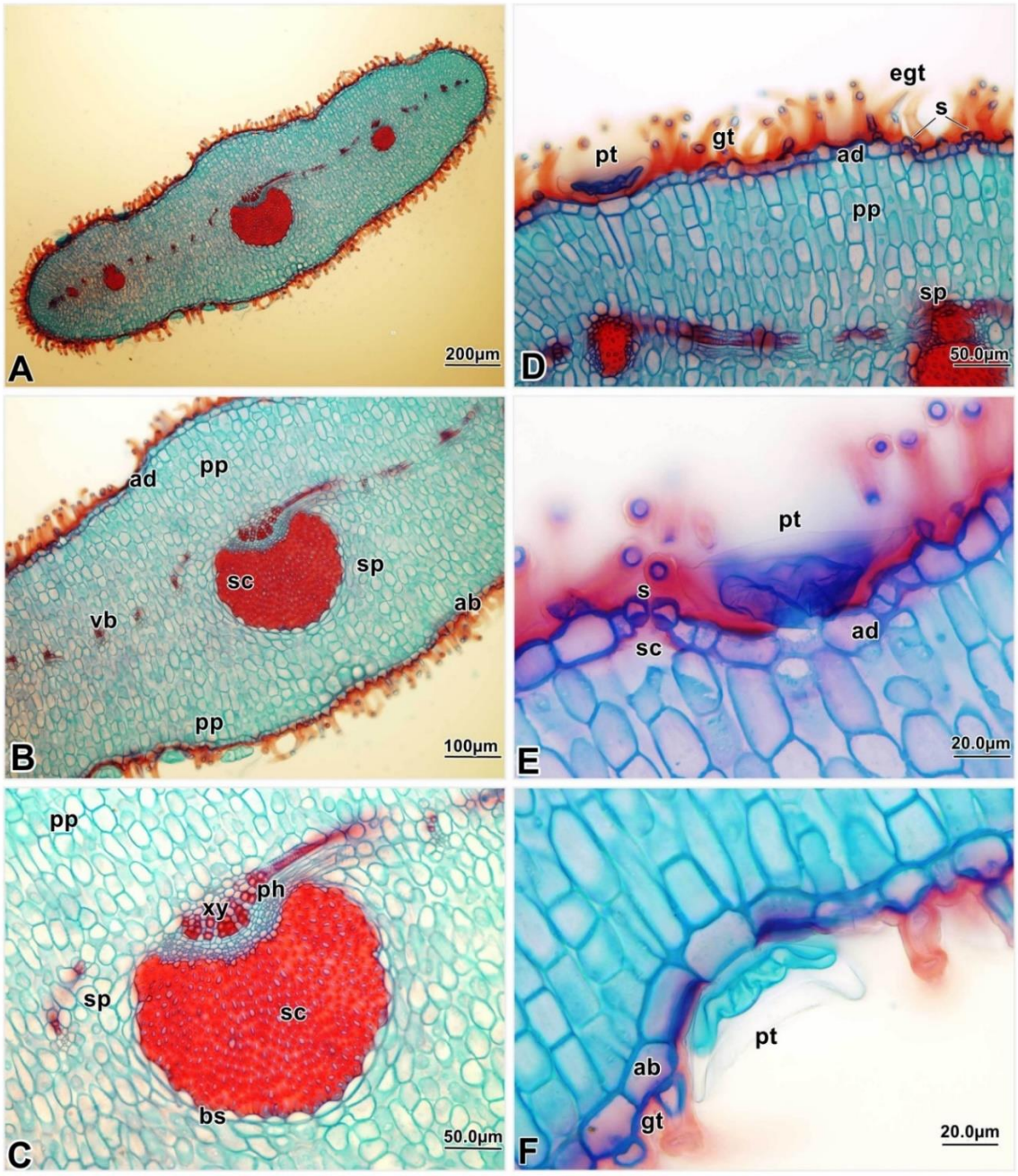

Figure 2. Photographs of a microscopic of the leaf cross-section of *T. spathulifolius*
ab: abaxial epidermis, ad: adaxial epidermis, bs: bundle sheath, egt: eglandular trichome, gt: glandular trichome, pp: palisade parenchyma, pt: peltate trichome, s: stomata, sc: stomatal cavity, sc: sclerenchyma, sp: spongy parenchyma, vb: vascular bundle, xy: xylem.

Fluorescence Analysis of Aerial Part Powder of Thymus spathulifolius

Fluorescence analysis is also an important analysis tool because it gives information about the compounds in the plant drug. The color of the plant extract is mainly due to its chemical compositions. The fluorescence analysis of the powder drug was conducted by mixing the sample with various chemical reagents and solvent, and the observations were carried out in visible light and UV light both with short and long wavelength. The results of fluorescence analysis of powder treated with different chemicals were shown in Table 2.

Table 2. Fluorescence analysis of aerial part powder of *T. spathulifolius*

No	Reagents	Day light	UV light (254nm)	UV light (366nm)
1	Powder	Whitish Green	Brown	Yellowish Brown
2	Powder + Water	Light Green	Whitish Yellow Fluorescence	Greenish Yellow
3	Powder + Methanol	Light Yellow	Blue	Light Brown
4	Powder + Ethylacete	Brown	Bluish Flourescence	Brownish Yellow Flourescence
5	Powder + chloroform	Brown	Blue	Yellowish Flourescence
6	Powder + n-hexane	Brown	Dark Green	Whitish Flourescence
7	Powder + CCI_4	Light Brown	Deep Violet	Whitish Flourescence
8	Powder +Xylene	Light Brown	Violet-Blue	Bluish Yellow
9	Powder + Conc. Sulphuric acid	Dark Brown	Brown	Dark Yellow
10	Powder + dil. Sulphuric acid	Whitish Green	Brown	Greenish Brown
11	Powder + Conc. Hydrochloric adid	Yellow	Dark Green	Brown
12	Powder + dil. Hydrochloric adid	Light Green	Dark Brown	Brown
13	Powder + Conc. Nitric acid	Orange	Blakish Brown	Brown
14	Powder + dil. Nitric acid	Light Yellow	Dark Brown	Light Brown
15	Powder + Acetic acid	Light Yellow	Navy Blue	Whitish Flourescence
16	Powder + Picric acid	Greenish Yellow	Greenish Blue	Bluish Green Flourescence
17	Powder + 1N NaOH	Yellow	Green Flourescence	Yellowish Green Flourescence
18	Powder + Ammonia	Dark Yellow	Black	Yellow
19	Powder + 5% Iodine	Light Yellow	Dark Brown	Blue
20	Powder + 5% $FeCI_3$	Green	Dark Brown	Black

Preliminary Phytochemical Studies

Phytochemical analysis is important for determining the quality of plant materials. Preliminary phytochemical analyses of *T. spathulifolius* extracts revealed the presence of flavonoids, steroids, phenolic compounds, tannins, and voletile oils, as shown in Table 3. In a previous study, luteolin, rosmarinic acid, vanilic acid, coumarin were determined as the major compounds in the extracts of *Thymus cariensis* and *Thymus clinics* (Küçükaydın et al., 2021).

Table 3. Qualitative phytochemical tests on extracts of aerial part of *T. spathulifolius*

Phytochemicals	Chemical test	Hexane extract	Chloroform extract	Ethylacetate extract	Methanol extract	Water extract
Carbohydrates	Molish's test	–	–	+	+	+
	Fehling test	–	–	+	+	+
Glycosides	Borntager	–	+	+	+	+
	Killer killani	–	+	+	+	+
	Baljet	–	–	–	–	+
Alkaloids	Dragendroff's test	–	–	–	–	–
	Mayer's test	–	–	–	–	–
Steroids	Salkowski test	+	+	+	+	+
Protein	Biuret test	–	–	–	–	–
	Millon's test	–	–	–	–	–
Flavonoids	Siyanidin	–	+	+	+	+
	NaOH	–	+	+	+	+
Coumarins	NaOH + UV	+	+	+	+	–
Saponins	Foam test	–	–	–	–	–
Fats	Filter paper spot test	–	–	–	–	–
Volatile oils	Sudan III + 70% ethanol	+	+	+	+	–
Tannin, phenolic compounds	5% $FeCI_3$	–	+	+	+	+
	Lead acetate	–	+	+	+	+
	Gelatin	–	+	+	+	–

Conclusion

The aim of this study is to reveal various pharmacognostic parameters, physico-chemical properties and anatomical characters of *Thymus spathulifolius*. The anatomical examinations given in this paper provides the first detailed defination of *T. spathulifolius*. When these definitions are compared with those of Metcalfe & Chalk's study (1957) and those of some other studies about *Thymus* taxa (Satıl et al., 2005; Alan & Koca, 2007; Berciu & Toma, 2008; Selvi et al., 2013), it is seen that there are many similarities. Metcalfe & Chalk (1957) reported that in the Labiatae family, the pith rays of the root are 2-12 rows, sometimes high and heterogeneous. In the literature, investigations on the root anatomy of Thymus are quite limited. Observations of the cross-section of root of *T. spathulifolius* showed that the medullary rays 1–2 layered, parenchymatic and heterogeneous. The primary xylem fills the middle of the root. Some researchers reported that in other Lamiaceae members, medullary (pith) rays are 1–10 layered and middle of the root is filled with primary xylem (Çobanoğlu, 1988; Özdemir & Senel, 1999; Baran & Özdemir, 2006; Özdemir et al., 2008; Temel et al., 2015).

According to this study observations, the stem is more or less angular shaped and collenchyma cells are located at the corners as other *Thymus* species. But, cork tissue below the endodermis was observed at only *Thymus migricus* Klokov & Des.-Shost. and *Thymus fedtschenkoi* Ronniger var. *handelii* (Ronniger) Jalas by Satıl et al. 2005. Metcalfe & Chalk (1957) noticed general anatomical structures as diagnostic values for Lamiaceae. It is a distinctive feature that the stem is rectangular in shape and consists of well-developed collenchyma groups covering a large area at four angles and a developed sclerenchyma tissue surrounding the vascular tissue.

Two different types of trichomes were observed on stems and leaves, glandular (consisting of peltate and capitate) and nonglandular trichomes. There are peltate hairs on the stem and leaves. These trichomes on the stem and leaves are of the Labiatae type. Kowalski et al. (2019) investigated in the epidermal cells of industrial species of Lamiaceae members, two types of Lamiaceae-type glandular trichomes were determined. First, short-and long-stalked capitate glandular trichomes with one- and two-cell secretory capitulum, and other peltate glandular trichomes with eight and more than a dozen-cell secretory capitulums. Metcalfe & Chalk (1957) stated that the coexistence of glandular and eglandular trichomes is a characteristic feature of Lamiaceae. The peltate trichomes have a same morphology, as well as the other types of capitate glandular and eglandular trichomes have been defined for the family (Metcalfe & Chalk, 1957; Werker, 1993). Endodermis of thin-walled cells often differentiated and composed of large cells with completely suberized walls. In *T. spathulifolius* leaves, phloem tissue is surrounded by thick sclerenchymatous tissue towards the lower epidermis. Same observations were defined in other *Thymus* species by Satıl et al. (2005), Alan & Koca (2007) and Selvi et al. (2013). According to Metcalfe & Chalk (1957), the continuous xylem is traversed by narrow medullary rays and xylem in the form of a continuous cylinder in the species of *Lavandula* L., *Micromeria* Benth., *Sideritis* L., *Teucrium* L. and *Thymus* species. Pith commonly homogenous, frequently becoming hollow in herbaceous species. In this study, observed that medullary rays are single rowed and numerous and center of the stem has pith cavity.

The good development of sclerenchyma, trichomes and essential oils are some of the xerophytic characters that increase the plant's ability to cope with drought. In these examinations, it was observed that the leaf and stem characteristics of T. spathulifolius were highly similar to the general anatomical features of Lamiaceae. Metcalfe & Chalk (1950) have reported the chlorenchyma in Lamiaceae often has isobilateral, dorsiventral or centric mesophyll organization. In this study isobilateral mesophyll was observed unlike as the dorsiventral type mentioned in *Thymus dacicus* Borbás and *T. glabrescens* Willd. (Berciu & Toma, 2008), *T. migricus* and *T. fedtschenkoi* var. *handelii* (Satıl et al., 2005), *T. pulvinatus* Čelak. and *T. cherlerioides* Vis. (Selvi et al., 2013) and *T.*

sibthorpii Benth., *T. sipyleus* Boiss., *T. leucostomus* Hausskn. & Velen. var. *argillaceus* Jalas, *T. longicaulis* C.Presl subsp. *longicaulis* var. *subisophyllus*, *T. longicaulis* subsp. *chaubardii* var. *chaubardii* (Borbás) Jalas (Alan & Koca, 2007). In the croos section of leaf, the sclerenchymatic tissue is well developed in vascular bundles surrounded by the bundle sheath.

Consequently, it can be said that there are some differences as well as similarities between other *Thymus* species and the *T. spathulifolius* in literature. The medullary rays one or two layered, parenchymatic, heterogeneous and center of the root is filled with primary xylem. There is 3-4 layered cork tissue below endodermis and secondary xylem is forming a continuous cylinder in stem of *T. spathulifolius.* The leaves are ecvifacial type and glandular and eglandular trichomes on the surface of both epidermis.

Quality control of raw drugs is very important. One of the tenets of herbal medicine is that the maximum effectiveness of the drug derives from the whole drug or its raw extract rather than from isolated compounds. It is necessary to comply with the quality control criteria specified for the drugs in order to obtain maximum benefit from the herbal medicines. Studies of physicochemical constants of drugs may be a rich source of information and are commonly used to assess purity and quality of drugs. Knowing the extractable amount with different solvents gives information about how much plant material should be collected to prepare the extract for future studies on this plant. From the study, the extractive value for water was highest than other solvents, that was followed by methanol. The ash value determines the contaminants or earchy matter present with drugs therefore it is important to evaluate the impurities of drugs These results can be used as appropriate quality control measures to ensure the quality, safety, and efficacy of these herbal medicines. This study may be helpful to preparation of monograph and herbal pharmacopeia standards of this plant. The phytochemical analysis indicate that this plant have phenolic compounds, flavonoids and carbohydrates. To our knowledge, this is first study of its kind on *T.spathulifolius*, therefore, it will be important to review and research on this plant.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: N.E.; Investigation: N.E., M.T.; Material and Methodology: N.E., M.T.; Supervision: M.T.; Visualization: Y.A.; Writing-Original Draft: N.E., Y.A.; Writing-review & Editing: M.T.; Other: Author has read and agreed to the published version of manuscript.

Conflict of Interest

The author has no conflicts of interest to declare.

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