

Evaluation of avocado (*Persea americana* Mill.) leaves in terms of public health

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ABSTRACT: *Persea americana* Mill. (Lauraceae) originated in Central and Southern of South America, is an evergreen tree. The tree is commonly known as avocado. Avocado is cultivated in all tropical and subtropical regions in the world. This tree has been usually grown in the southern coastal region of Turkey due to its commercial importance. Its fruit is a drupe which consumed as food. Except this, the leaves are widely used for pass kidney stone and against the urinary tract infections as therapeutic among the people in Turkey and Cyprus.

The present study, 13 different samples from 5 diverse cities (Ankara, Hatay, İstanbul, Kayseri and Aydın) were purchased. The sample to be used as standard was obtained from the culture form (Dörttyol, Hatay). The morphological properties of leaves, which are part of the plant used as a drug, were determined both in the standard sample and in the purchased samples. Anatomical features of cross and surface sections from standard avocado leaves with cross sections from the petiole of natural avocado leaves were investigated and determined. Besides, the distinctive anatomical structures of the powdered samples (standard sample and samples purchased from the market) were demonstrated. The avocado leaf is bifacial. The hairs are only located the lower surface of the leaf and mostly unicellular. Secretory cells and crystals were observed in the avocado leaf and petiole. Crystals are simple in various sizes and small raphids. The stomata confined to the lower surface and anomocytic type (3-6 subsidiary cells). In cross section taken from the petiole, the hairs were observed to be gathered on the upper surface of the petiole.

KEYWORDS: *Persea americana*; Lauraceae; morphology; anatomy; powder drug; public health; traditional use.

1. INTRODUCTION

The genus *Persea* Mill. includes about 150 species of tropical evergreen trees. *Persea* name has been came from ancient Greek name of an Egyptian tree with sweet fruits originated propably from Perseus. *Persea americana* Mill. (Lauraceae) originated in Central and Southern of South America, is a shiny evergreen tree, known as "avocado". *P. americana* is a medium to large tree (9-20 m in height), much more branched, vigorous and round topped [1, 2]. Avocado is cultivated in all tropical and subtropical regions in the world for its delicious and highly nutritious fruit. The leaves are 6-30 cm in length and 3.5-19 cm in wide and narrow to broadly elliptical in shape. They are constantly pubescent and reddish when young. When they mature, becoming smooth, leathery and dark green. Its fruit is consumed as food. *P. americana* is cultured in the southern coastal region of Turkey due to its commercial importance. The leaves are widely used for pass kidney stone and against the urinary tract infections as therapeutic among the people in Turkey [1, 3-6]. Besides these, they have been used internally as an infusion for diarrhea, stomachache, body pain, headache, wounds, fever, heart attack, osteoporosis, vomit, sore throat, to keep away from bad spirits in Mexico and as a decoction for high blood pressure in Caribbean islands [7, 8]. The fresh leaves have been consumed in the form of aqueous infusion or decoction for influenza, bronchitis, menstruation pain, diabetes, rheumatism and also externally hair tonic in Ecuador and as well as the leaves have been employed as antimalarial in Nigeria [9, 10].

Especially, essential oils and flavonoids were reported to be present in *P. americana* leaf [11-15]. Also, avocado leaves contain persin which is a toxin for lactating livestock [16]. *In vitro* activity studies such as

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anticancer, anticholinesterase, antimicrobial, antioxidant, antityrosinase have been performed on the leaves [11, 14, 17-23]. *P. americana* leaf has exhibited pharmacological activities such as analgesic, anti-inflammatory, anticonvulsant, antihyperlipidemic, hypocholesterolemic, hypoglycemic, hypotensive properties [24-28].

In this study, samples from different cities of Turkey sold with the name of "avokado yaprağı" in the market were taken and their morphological characteristics, anatomical structures and purities were investigated compared to the standard sample.

2. RESULTS

2.1. The usage and sold

It was determined that the samples sold in the market under the name of "avokado yaprağı" were generally sold in open conditions (Aydın, Hatay, İstanbul, Kayseri, Nicosia), but they were mostly sold in packages on Ankara. It has been determined that the drug are used as decoction for kidney and urinary tract inflammation, bile and kidney stones and edema in the direction of findings obtained from the public. Also, it has been observed that the drug are used as diuretic in Cyprus. Findings related to the use of the drug were found to be consistent with the source data [6-10].

2.2. Morphological Results

The leaves are simple, petiolate, elliptical to lanceolate, green, often pubescent, about 9-16.5 cm in length and 4.5-9 cm in width. The margin is entire. The lower surface is paler than the upper surface and midrib and veins more prominent below. The apex is usually acute, rarely acuminate; the base is usually acute with pinnate reticulate venation (Figure 1C, D). Findings obtained this study have found to be consistent with the data sources [1, 29]. Morphological findings obtained from the examined samples which are given in Table 1 and Figures 2, 7. It was observed that the samples were generally composed of pure leaves, and fragmented. Mouldy and insect infested leaf samples were found in some samples (Figures 2B, 7A2, 7A3, 7C2, 7D2, 7E2). However, with not being too high, parts of other plants and foreign matter (stone and nylon pieces, etc.) were also identified as impurities (Figures 2A, 7).

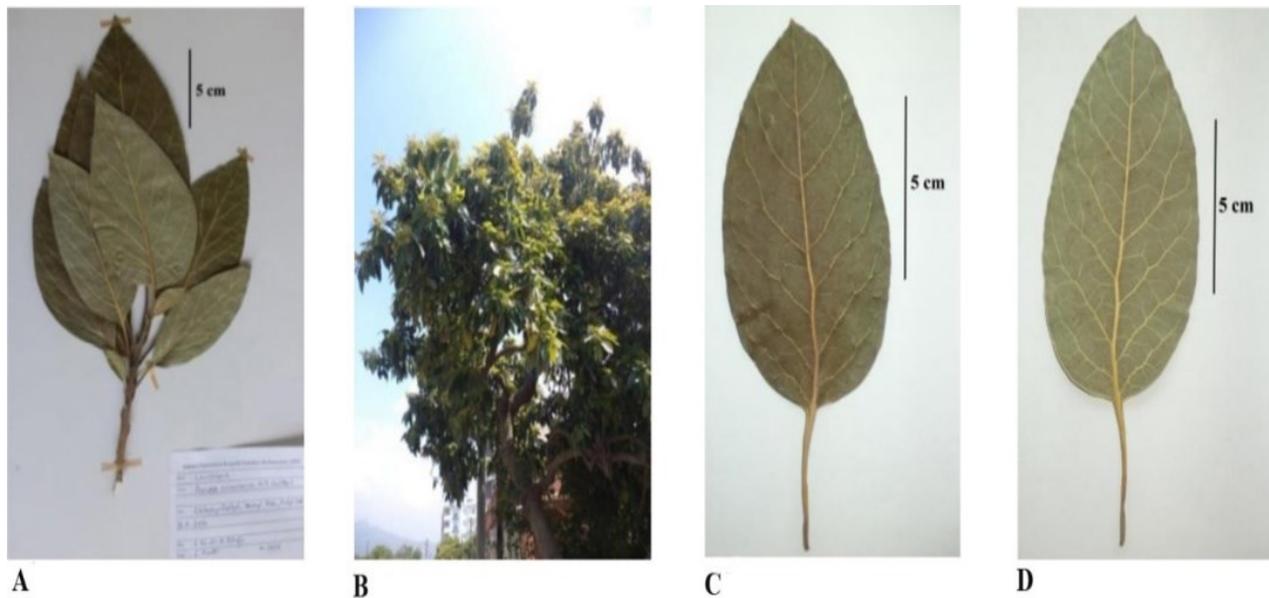


Figure 1. *Persea americana* A. Herbarium sample, B. Cultivated sample, C. Leaf upper surface, D. Leaf lower surface.

Table 1. The morphological characteristics of drug samples used in the study.

Sample	General appearance	Color	Leaf shape	Proportion of non-drugs items
A1	Generally obsolete and fragmented leaves	Green, brownish	Undetectable	1% branch pieces, 1% foreign matter (stone)
A2	Generally fresh and fragmented leaves but obsolete and mouldy leaves available	Green, brownish	Undetectable	1% branch pieces
A3	Generally fresh and fragmented leaves but obsolete and mouldy leaves available	Green, brownish	Undetectable	1% branch pieces, 1% other plants parts
AY	Generally fresh leaves	Green	Elliptical	-
D1	Generally fresh and fragmented leaves	Green	Undetectable	-
D2	Generally fresh and fragmented leaves, there are also leaves eaten by insects	Green	Undetectable	1% branch pieces
D3	Generally obsolete and fragmented leaves	Green, brownish	Undetectable	1% branch pieces
I1	Generally fresh and fragmented leaves	Green, brownish	Elliptical	1% branch pieces, 2% other plant parts
I2	Generally fresh and fragmented leaves, mouldy leaves available	Green, brownish	Elliptical	-
I3	Generally fresh and shredded leaves	Green, brownish	Undetectable	1% other plant parts, 1% foreign matter (nylon pieces)
K1	Generally fresh and fragmented leaves	Green	Undetectable	1% branch pieces
K2	Generally fresh and fragmented leaves, there are infested leaves by insect	Green	Elliptical	1% branch pieces
K3	Generally fresh and fragmented leaves	Green, brownish	Elliptical	1% branch pieces, 1% other plant parts, 1% foreign matter (nylon pieces)

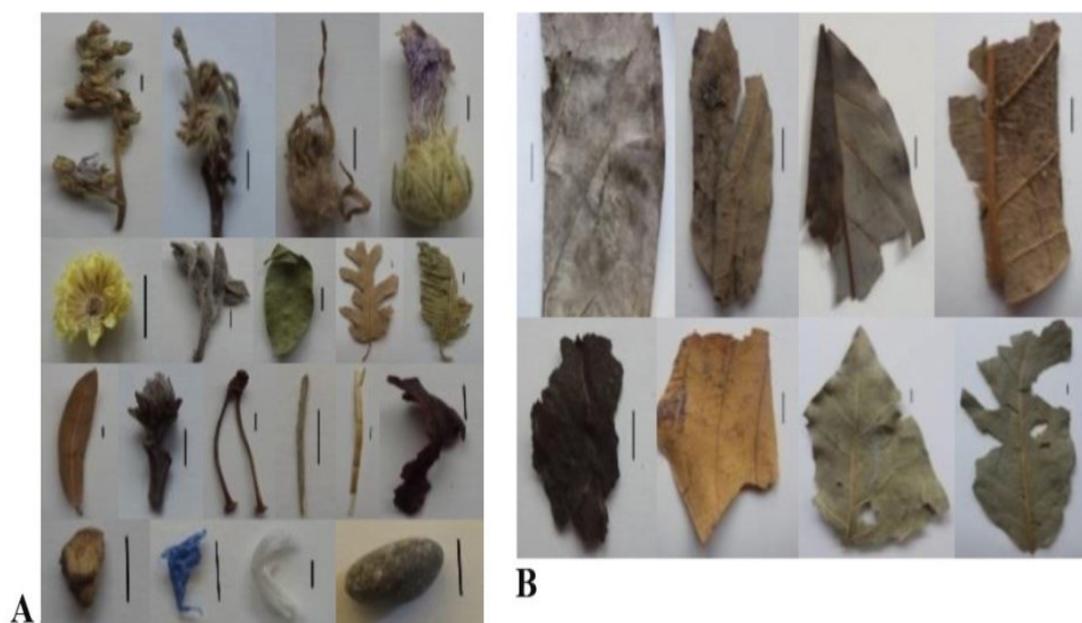


Figure 2. A. Parts of other plants and foreign matter observed in drug samples (scale 5 mm), B. Mouldy and insect infested leaf samples (scale 5 mm).

2.3. Anatomical Results

2.3.1. Cross section of the leaf midrib

Leaf is hypostomatic and bifacial. The upper and lower epidermis are uniseriate and the lower epidermal cells are larger and bigger than the upper epidermal cells, covered by a thick cuticle. The lower epidermis cells have beaded walls (Figures 3A, 3B, 3C). Palisade parenchyma, 1-2 layered, cells are long cylindrical. Spongy parenchyma with large intercellular spaces is located under the palisades. A few layers of collenchyma and colourless parenchyma cells are located beneath in the epidermis in the midrib region, and palisade and sponge parenchyma are not visible in this region. The vascular bundle is composed of the phloem on the outside and the xylem on the inside, and surrounded by a sclerenchymatous sheath. The covering trichomes are simple, mostly unicellular and thick walled. Secretory cells containing oil, and crystals in various sizes, small raphids and abundant starch were observed in especially parenchymatic cells (in the midrib region) (Figure 3).

2.3.2. Surface section of the leaf upper epidermis

The upper epidermis is composed of irregularly shaped cells with weightlessly sinuous walls. Below each epidermal cell, palisade parenchyma cell with a generally rounded shape ranging from 2 to 10 was seen. The hairs were not observed (Figure 4A).

2.3.3. Surface section of the leaf lower epidermis

The lower epidermis consists of irregularly shaped cells with significant sinuous walls. Stomata are anomocytic with 3-6 subsidiary cells. The simple, unicellular covering trichomes were found intensely (Figure 4B).

2.3.4. Cross section of the petiole

Epidermal cells are generally rectangular in a single layer with covered thick cuticle layer. Under the epidermal cells, there are multi-layers of collenchyma. The vascular bundle is located within parenchyma cells under collenchyma. Around the xylem and phloem is surrounded by a crescent-shaped of sclerenchymatous bundle. The covering trichomes, unicellular with thick walled, were determined to be collected especially on the upper surface of the petiole. Oil cells, abundantly crystals in various sizes, small raphids and starch were determined (Figure 5).

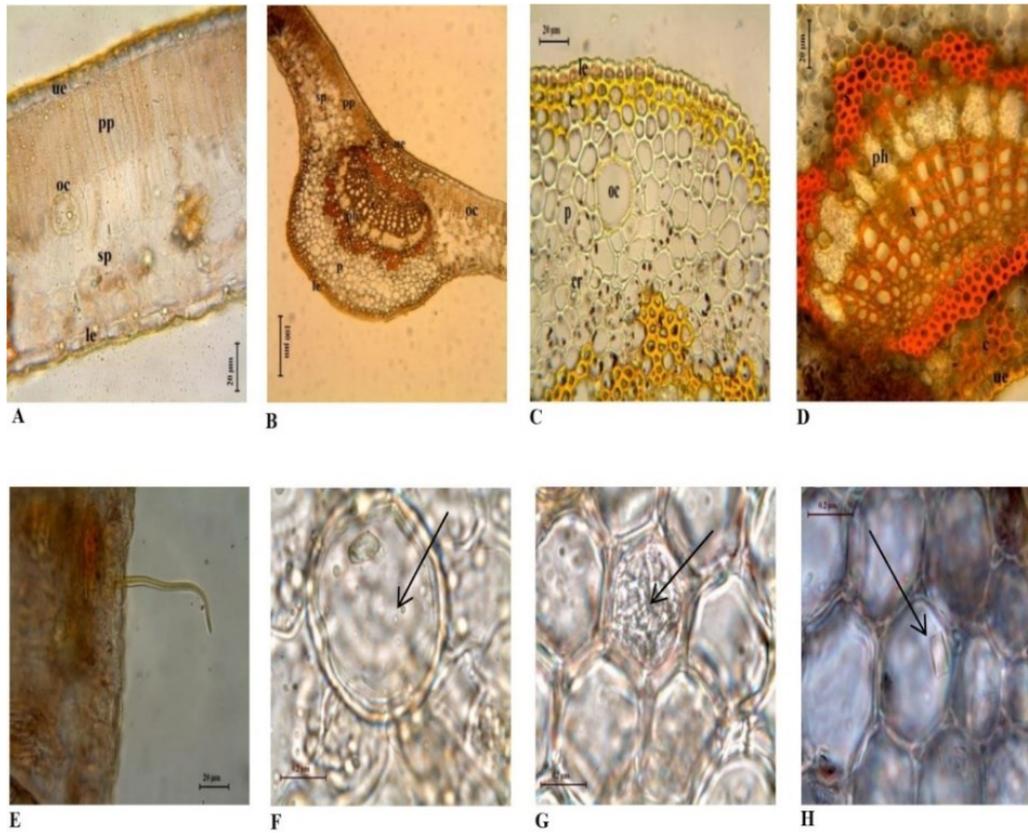


Figure 3. Cross section of leaf midrib of *Persea americana* **A.** Cross section of leaf blade, **B.** Leaf midrib and leaf blade, **C.** Leaf midrib, **D.** Vascular bundle, **E.** Covering trichome, **F.** Oil cell, **G.** Crystals in shape of small needles, **H.** Prismatic crystal.

ue: upper epidermis, le: lower epidermis, c: collenchyma, p: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, ph: phloem, x: xylem, s: sclerenchyma, cr: crystal, oc: oil cell.

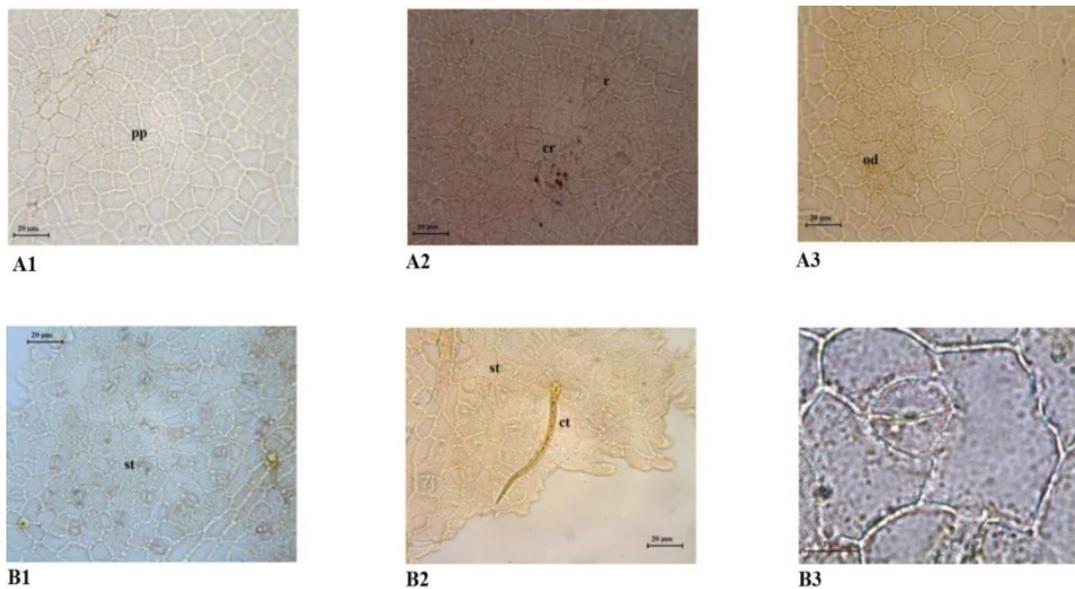


Figure 4. **A.** Surface section of leaf upper epidermis of *P. americana* (**A1.** Upper epidermis and palisade parenchyma, **A2.** Upper epidermis, crystals and raphids, **A3.** Upper epidermis and oil drops), **B.** Surface section of leaf lower epidermis of *P. americana* (**B1.** Lower epidermis and stoma, **B2.** Lower epidermis and covering trichome, **B3.** Stoma). pp: palisade parenchyma, cr: crystal, r: raphids, od:oil drops, st: stoma, ct: covering trichome.

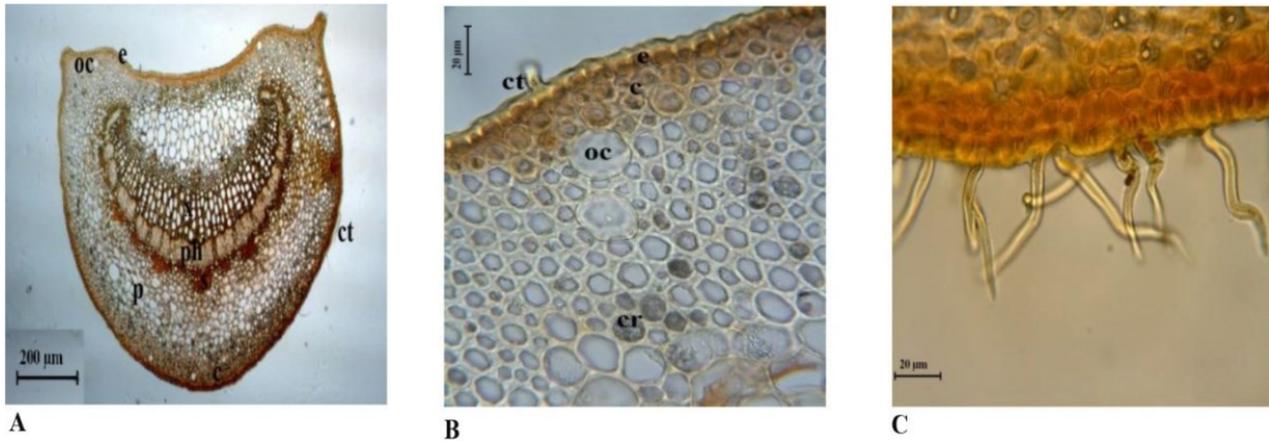


Figure 5. Cross section of the petiole of *P. americana* **A.** General view, **B.** Collenchyma, oil cells and crystals, **C.** Covering trichomes.

e: epiderma, c: collenchyma, ph: phloem, x: xylem, s: sclerenchyma, p: parenchyma, cr: crystal, oc: oil cell, ct: covering trichome.

2.3.5. Examination of the powdered drug

It was determined that all of the examined powdered drugs are fibrous, heterogeneous, in dark green colour. All samples were found to have an anise-like odour and a spicy taste. Also, the characteristic anatomical elements of samples were defined and illustrated in the Figure 6.

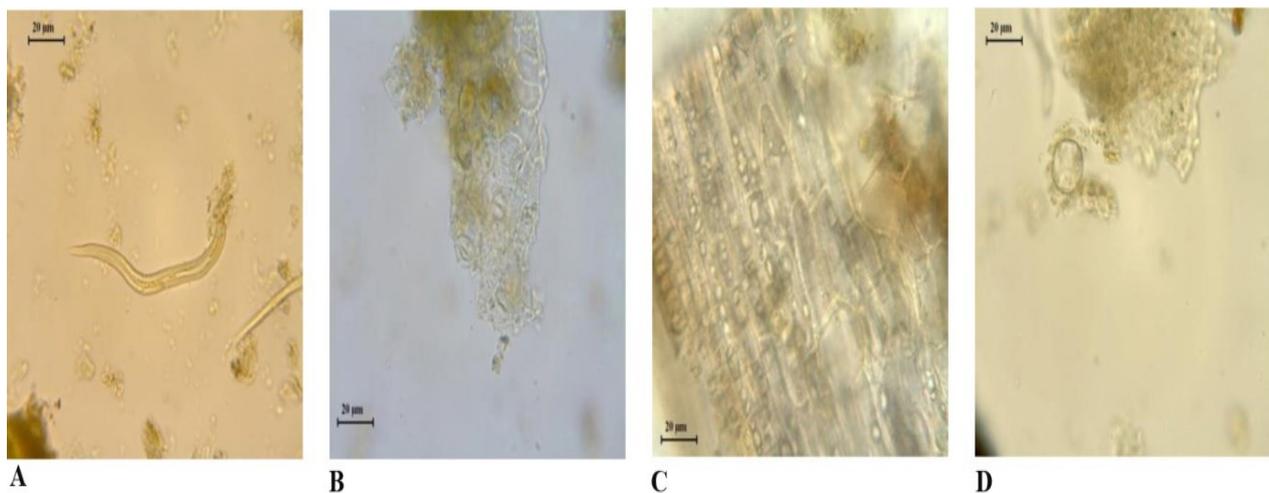


Figure 6. Microscopic properties of powdered drug samples of *P. americana* **A.** Covering trichome, **B.** Stoma, **C.** Crystals, **D.** Oil cell.

3. DISCUSSION

This study was made to determine if they have scientific drug characteristics which used for its therapeutic feature in folk and supplied from the market of avocado leaves. The drug known as “avokado yaprağı” has been found to be usually sold as open. Morphological studies have shown that the samples are usually composed of pure leaves. Mouldy and insect infested leaf samples were found in the A2, A3, D2, I2 and K2 locations. AY, D1 and I2 locations are completely pure. The branches pieces of the plant were determined in almost all samples (1 %). Foreign matter presence in A1, I3 and K3 locations (stone and nylon pieces, etc.) was also determined. In addition to being not very high, the presence of other plants parts was also determined (Table 1). According to European Pharmacopoeia (2010) [30], herbal drugs should be free from moulds, insects and other animal contamination.

Anatomical examinations revealed the following findings: The leaf is bifacial. The covering trichomes, thick walled and mostly unicellular, are only located lower surface of the leaf, however, they are gathered partially on the upper surface of the petiole. Secretory cells, crystals in various sizes, small raphids were

observed in the avocado leaf and petiole. The stomata occur only on lower epidermis of the leaf and anomocytic with 3-6 subsidiary cells (Figures 3-5). In addition, no different findings were observed in the powdered drug samples purchased from the market and standard sample. Obtained findings found to be generally consistent with the literature. Metcalfe and Chalk (1965) [31] reported cells of the lower epidermis were papillose and stomata were rubiaceous. Also, they mentioned the presence of hypoderm and mucilage cells in *Persea*. Mahmoud et al. (2016) [29] recorded thin cuticle layer and the presence of hairs on upper epidermis of the leaf. However, these anatomical features were not observed during the our anatomical examinations.

4. CONCLUSION

It has been determined that all of the samples presented to the sale are morphologically and anatomically formed from the correct drug, namely *P. americana* leaves. However, since the European pharmacopoeia [30] (2010) does not allow animal contaminations in herbal drugs, the samples do not carry the proper conditions for herbal drug description. In addition, the sales conditions of the samples were not considered suitable for human health.

5. MATERIALS AND METHODS

13 different samples were purchased from 5 different cities including İstanbul (3), Ankara (3), Kayseri (3), Hatay (3) and Aydın (1) and, standard sample was obtained from the culture form (AEF 26915) (Table 2). Information about use of the plant was received, but the sample was not purchased in Nicosia (North Cyprus). The morphological properties and purities of all samples were examined and photographed (Sony Cyber-shot DSC-S5000). In the anatomical study, cross and surface sections from standard avocado leaves with cross sections from the petiole were examined with Sartur reagent [32] under the microscope and the images on the light microscope were taken with a Leica DM 4000 B camera. Besides, the distinctive anatomical structures of the powdered samples (standard sample and purchased samples from the market) were demonstrated.

Table 2. The locations of the samples which used in the study.

Location	Sample	Figure number
Standard	S Cultured form from Dörtyol -Hatay (AEF 26915)	Fig. 1
Ankara 1	A1	Fig. 2A1
Ankara 2	A2	Fig. 2A2
Ankara 3	A3	Fig. 2A3
Aydın	AY	Fig. 2B
Dörtyol 1 (Hatay)	D1	Fig. 2C1
Dörtyol 2 (Hatay)	D2	Fig. 2C2
Dörtyol 3 (Hatay)	D3	Fig. 2C3
İstanbul 1	I1	Fig. 2D1
İstanbul 2	I2	Fig. 2D2
İstanbul 3	I3	Fig. 2D3
Kayseri 1	K1	Fig. 2E1
Kayseri 2	K2	Fig. 2E2
Kayseri 3	K3	Fig. 2E3

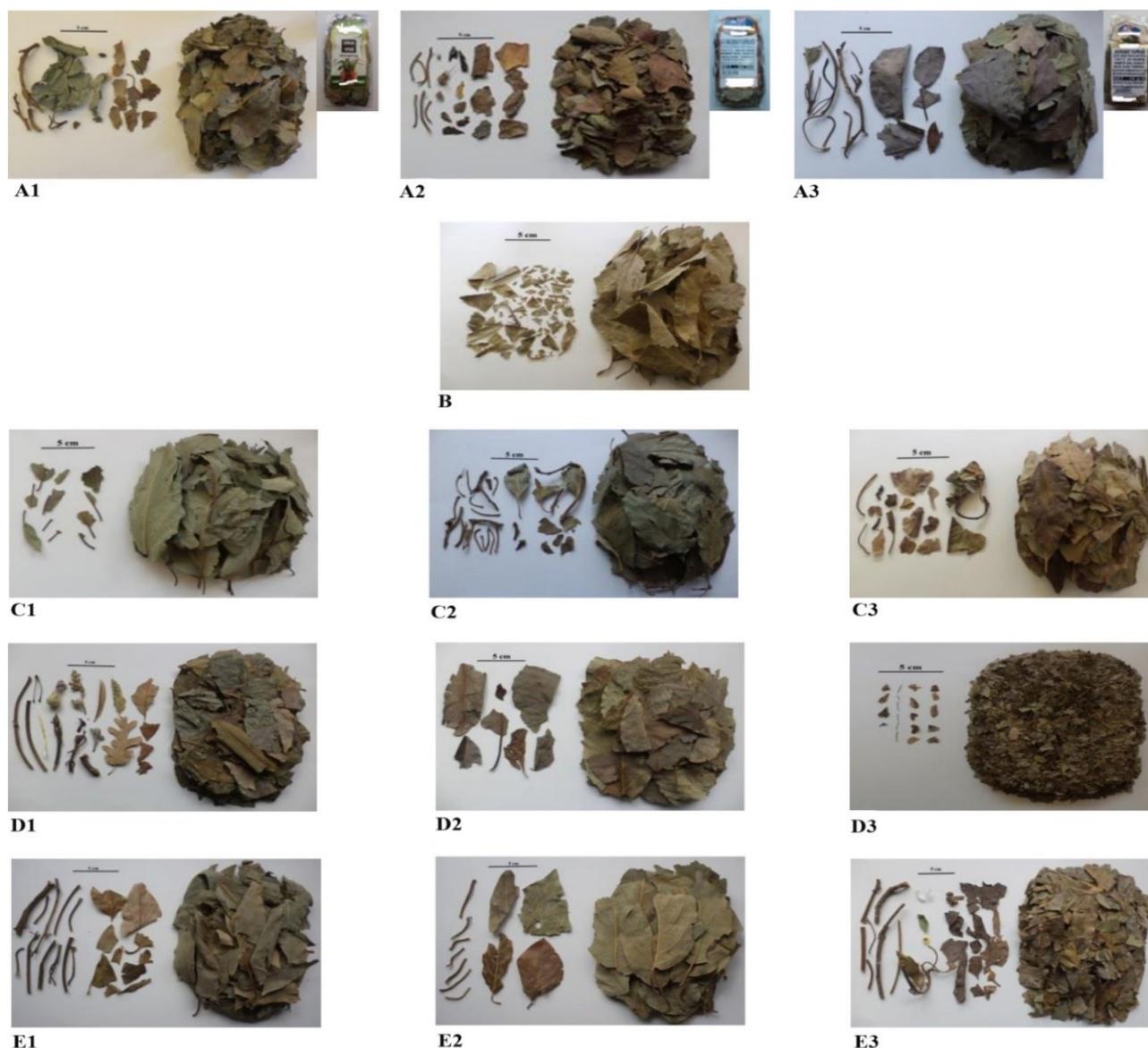


Figure 7. A. Avocado samples obtained from markets in Ankara (A1. Sample 1, A2. Sample 2, A3. Sample 3), B. Avocado samples obtained from market in Aydın, C. Avocado samples obtained from markets in Hatay (C1. Sample 1, C2. Sample 2, C3. Sample 3), D. Avocado samples obtained from markets in İstanbul (D1. Sample 1, D2. Sample 2, D3. Sample 3), E. Avocado samples obtained from markets in Kayseri (E1. Sample 1, E2. Sample 2, E3. Sample 3).

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