J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 50(2): 301-315, 2026
Doi: 10.33483/jfpau.1758042
Research Article / Araştırma Makalesi

PHARMACOPOEIA ANALYSIS OF PIPERIS LONGI FRUCTUS SAMPLES PURCHASED FROM THE MARKET

PİYASADAN TEMİN EDİLEN PİPERİS LONGİ FRUCTUS ÖRNEKLERİNİN FARMAKOPE ANALİZİ

Melek KARAASLAN[1], Burçin ERGENE[1]*, Funda YAVUZ[2], Gülcan İlayda DİLER[2]

[1]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye
[2]Ankara University, Faculty of Pharmacy, Ankara, Türkiye

ABSTRACT

Objective: *Piperis longi fructus is an upright and slender vine with smooth, green, and thin stems. Its fruits, which are used as drug, are fleshy and oval. It is commonly used as a spice, in pickles, foods, and beverages. The plant contains various chemical compounds, including essential oils, saponins, cyanogenic glycoside known as amygdalin, and primarily the alkaloid piperine. In traditional medicine systems, it is used for respiratory infections, muscle pain, insomnia, epilepsy, and many other conditions. Due to its broad usage and strong effects, careful attention must be given to its consumption; otherwise, it may pose risks to public health. Therefore, the analysis of this herbal drug should be conducted meticulously. This study aims to investigate the compliance of Piperis longi fructus samples purchased from the market with the standards specified in the Turkish Pharmacopoeia 2017.*

Material and Method: *In this study, Piperis longi fructus samples obtained from 12 different herbalists were analyzed according to the monograph in the Turkish Pharmacopoeia (2017). The analysis included morphological examination, microscopic analysis, and thin-layer chromatography. Additionally, the determination of foreign matter, loss on drying, and total ash content was carried out. Finally, quantitative determination was conducted using high-performance liquid chromatography.*

Result and Discussion: *As a result of the pharmacopoeial analysis conducted within the study, none of the twelve Piperis longi fructus samples fully complied with the pharmacopoeial standards.*

Keywords: *Long pepper, pharmacopoeial analysis, Piperis longi fructus, Turkish Pharmacopoeia*

ÖZ

Amaç: *Piperis longi fructus, düzgün, yeşil ve ince gövdeye sahip, dik ve ince yapılı bir sarmaşık türüdür. Drog olarak kullanılan meyveleri etli ve oval yapıdadır. Yaygın olarak baharat, turşu, yiyecek ve içeceklerde kullanılmaktadır. Bitki; uçucu yağlar, saponozitler, amigdalin olarak bilinen siyanogenetik heterozit ve başlıca piperin alkaloidi olmak üzere çeşitli kimyasal bileşikler içermektedir. Geleneksel tıp sistemlerinde solunum yolu enfeksiyonları, kas ağrısı, uykusuzluk, epilepsi ve daha birçok rahatsızlıkta kullanılmaktadır. Yaygın kullanımı ve güçlü etkileri nedeniyle tüketimine dikkat edilmelidir; aksi takdirde halk sağlığı açısından risk oluşturabilir.*

* **Corresponding author:** Burçin Ergene
E-mail: ergene@pharmacy.ankara.edu.tr
Submitted date: 04.08.2025 **Accepted date:** 27.02.2026 **Published date:** 19.05.2026
Citation: Karaaslan, M., Ergene, B., Yavuz, F., Diler, G.İ. (2026). Pharmacopoeia analysis of piperis longi fructus samples purchased from the market. Journal of Faculty of Pharmacy of Ankara University, 50(2), 301-315.

Bu nedenle, bu bitkisel droğun analizleri titizlikle yapılmalıdır. Bu çalışma, piyasadan temin edilen Piperis longi fructus örneklerinin Türk Farmakopesi 2017'de belirtilen standartlara uygunluğunu araştırmayı amaçlamaktadır.

Gereç ve Yöntem: *Bu çalışmada, 12 farklı aktardan temin edilen Piperis longi fructus örnekleri, Türk Farmakopesi (2017) monografına göre analiz edilmiştir. Analizler; morfolojik inceleme, mikroskobik analiz ve ince tabaka kromatografisini içermektedir. Ayrıca, yabancı madde, kurutmada kayıp ve toplam kül miktarı tayini gerçekleştirilmiştir. Son olarak, yüksek performanslı sıvı kromatografisi yöntemiyle kantitatif analiz gerçekleştirilmiştir.*

Sonuç ve Tartışma: *Çalışma kapsamında yapılan farmakope analizleri sonucunda, on iki adet Piperis longi fructus örneğinin hiçbirinin farmakope standartlarına tam olarak uygun olmadığı belirlenmiştir.*

Anahtar Kelimeler: *Farmakope analizi, Piperis longi fructus, Türk Farmakopesi, uzun biber*

INTRODUCTION

Since the beginning of human history, medicinal plants have been used to treat various ailments. Herbal medicine, which dates back to the origins of human existence, has its roots in China and India [1-3]. Traditional medicine is widely used in all regions of the developing world and has rapidly spread in industrialized countries. According to the World Health Organization, traditional medicine is widely practiced in both underdeveloped countries within Africa and some developing countries. In Africa, the ratio of traditional medicine practitioners to the population is 1:500, while for medical doctors, this ratio increases to 1:40000. Due to cultural and historical influences, even in highly developed healthcare systems such as those in Singapore and the Republic of Korea, traditional treatments are used at rates between 76% and 86%. Additionally, traditional medicine is widely practiced in North America and many European countries [4].

Research on natural herbal medicine products is an essential approach for discovering and developing biologically active compounds with unique structures and mechanisms of action. Although their use belongs to traditional treatment regimens, plant-derived compounds still constitute a significant portion of pharmaceutical agents today [5].

Piper longum L. is an important medicinal plant belonging to the Piperaceae family. This family consists of approximately 100 species, including well-known varieties such as Piper nigrum L. (black pepper) and Piper betle L. (betel vine). Other wild species of this genus with medicinal value, sold under the name "long pepper," include Piper chaba W. Hunter, Piper peepuloides Roxb., Piper mullesua Buch.-Ham. ex D.Don, and Piper hapnium Buch.-Ham. ex Hook.f. [6].

P. longum is native to South and Southeast Asia and is widely cultivated in India. It is also found in Sri Lanka, Burma, Malaysia, Nepal, Singapore, and other South Asian countries [7].

P. longum is a climbing plant with slender, upright branches, smooth green stems, and a creeping habit. It is a dioecious, perennial, deciduous shrub. Its creeping branches spread across the ground, while its fruit-bearing branches grow upright. The young branches have fine, multicellular hairs, but the older branches are completely hairless. The creeping stems are connected to the fruiting branches. The leaves are simple, petiolate or sessile, distinctly dimorphic, 5-9 cm long, 3-5 cm wide, ovate, and broadly cordate at the base. The fruits are oval, fleshy, embedded in the spike, and turn from green to black upon ripening. Male plants have longer, slender spikes, while female spikes are 1.3-2.5 cm long and 4.5 mm in diameter. Flowering occurs throughout the year, and the flowers are unisexual. The clustered flowers form small gray fruit clusters [6,8].

The fruits of *P. longum* contain essential oils, starch, protein, alkaloids, saponins, carbohydrates, and cyanogenic glycoside (amygdalin). The primary chemical components are piperine alkaloids, piperlongumine, piperlonguminine, and methyl-3,4,5-trimethoxycinnamate [9]. The fatty acids identified in crushed seeds include palmitic, hexadecenoic, stearic, linoleic, oleic, arachidic, and behenic acids which are highly saturated. The roots contain bractistin, pipersitin, piperderidine, longamide tetrahydropiperine, tetrahydropiperlongumine, dehydropipernonaline, piperidine, piperine, tetrahydropiperlongumine, trimethoxycinnamoyl piperidine, and piperlongumine. The essential oil of *P. longum* fruit consists of three main components; caryophyllene, pentadecan (each approximately 17.8%) and bisabolene (11%) [10].

In addition to being used as a spice, *P. longum* is used in pickles, food, beverages, liquor, and medicine. Since long pepper has a more intense aroma than black pepper, it should be used with caution unless a very strong flavor is desired. Unlike black pepper, it does not contain terpenic compounds, so it does not provide the same aroma and cannot be used as a direct substitute [11].

The most significant use of *P. longum* is in traditional medicine systems such as Ayurveda, Unani, and Siddha. Both its fruit and dried roots are utilized. The preparation of "piplamool" in these traditional medicine systems includes not only the slender roots but also thick stems and roots. The slender root is considered beneficial for digestion, as a laxative, an anthelmintic, a carminative, and an appetite stimulant. It is used to treat bronchitis, abdominal pain, spleen disorders, and tumors, as well as to reduce bile secretion. The fruit and roots have numerous medicinal uses. They are employed in treating respiratory conditions like cough, bronchitis, and asthma. When applied topically, they act as an analgesic for muscle pain and inflammation. They are used as a snuff for coma and lethargy, as a carminative when taken orally, as a sedative for insomnia and epilepsy, as a general tonic and anti-anemic agent, as a cholagogue, emmenagogue, and abortifacient. After childbirth, it is used to control postpartum bleeding, and root infusions are prescribed to expel the placenta [7,9].

A published study indicated that the alcoholic extract of Piperis longi fructus reduces transglutaminase activity, affects transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, and bilirubin levels, and improves liver function. It also has positive effects on the treatment of hepatitis B infection [12].

A study found that piperine enhances the bioavailability of various drugs such as vasicine, indomethacin, and diclofenac sodium, by modulating cell membrane dynamics, allowing the drugs to penetrate the intestinal epithelial barrier more effectively. The study also demonstrated that piperine increases serum concentration, absorption rate, and bioavailability of curcumin in both rats and humans [13].

To evaluate the dose and toxicity potential of Piperis longi fructus in vivo, a single daily oral dose of 3-5 g/kg was administered for acute toxicity tests and over 90 days in chronic toxicity tests. As a result of the tests, no side effects were observed. The LD_{50} values for piperine, piperlongumine, and piperlonguminine were found to be 56.2 ± 3.0, 110.1 ± 7.8, and 115.3 ± 9.5 mg/kg body weight, respectively. Acute toxicity of the studies was not observed at the doses of 3-5 g/kg. This study is significant for the determination of the appropriate usage dose and potential toxicity of Piperis longi fructus. However, due to potential interactions, *P. longum* fruits should be used with caution during pregnancy and breastfeeding [8].

In the content of this study, the compliance of twelve samples of Piperis longi fructus, purchased from the market, to the standards specified in the Turkish Pharmacopoeia 2017 was evaluated. To the best of our knowledge, this is the first study to evaluate the pharmacopoeial compliance of Piperis longi Fructus samples available on the Turkish market.

MATERIAL AND METHOD

Study Material

For the pharmacopoeia analysis of Piperis longi fructus, twelve different samples were obtained from various herbalists. To prevent any influence on the study results, each brand was numbered from 1 to 12. The analysis results were interpreted without considering the source of the samples. The pharmacopoeia analyses of the samples were conducted based on the Turkish Pharmacopoeia 2017 [14]. According to the TF 2017 monographs, the required tests for Piperis longi fructus include:

- Organoleptic control
- Microscopic examination
- Thin Layer chromatography
- Foreign matter detection
- Loss on drying
- Piperine quantification by liquid chromatography

Organoleptic Control

According to the Turkish Pharmacopoeia 2017, Piperis longi fructus samples are cylindrical or irregularly cylindrical, measuring 10-25 mm in length (rarely exceeding 25 mm) and 3-5 mm in diameter. They are blackish-brown to almost black in color. The spikes are dense and firm, composed of small fruits that are tightly fixed on the receptacle in regular or oblique rows. The berry-like fruits are spherical, approximately 1 mm in diameter. The bracts are black and spotted, situated between the depressions of adjacent berry-like fruits. Remnants of the flower stalk may be present at the base of the cylinder. The spikes are easily breakable, with an irregular and granular fracture surface. All samples were evaluated based on these criteria [14].

Microscopic Examination

According to the microscopic elements given in the monograph "Piperis longi fructus", the powder is gray or light brown in color. It is examined under a microscope (40x lens). In addition to the chloral hydrate solution recommended by the pharmacopoeia, Sartur reagent was also employed to enable clearer observation of certain elements. using chloral hydrate solution R and sartur R as reagents. The characteristic elements of the powdered material given in the monograph are as follows: fragments of the endocarp, consisting of sclereids, more or less elongated, approximately 75 µm long, pitted, with irregularly thickened walls and wide channels, associated with the reddish-brown pigmented layer of the testa with indistinct cells and also associated with the inner testa with rectangular, brown, thin-walled cells; fragments of the endocarp [transverse section, showing sclereids with irregularly thickened inner walls on the three lower sides usually associated with the testa (namely the pigmented layer with indistinct cells and the layer of the inner testa)]; fragments of the parenchyma of the mesocarp containing more or less polygonal sclereids, isolated or in groups, and oil cells about 50 µm in diameter; numerous ovoid or polygonal cells of the parenchyma of the seed; fragments of the epicarp with thin-walled cells; rare fragments from the center of the spike, consisting of parenchymatous cells and elongated sclereids up to 400 µm long; a few fragments of vascular tissue with spiral or striated vessels and fibers. Starch is visible as rounded, compound granules, each about 20 µm in diameter, made up of tiny individual granules, ovoid or polyhedral by compression, free or included in the parenchymatous cells of the seed [14].

Thin Layer Chromatography

Test solution was prepared by using 0.5 g of the powdered herbal drug. 5 ml of methanol R was added, and the mixture was sonicated for 10 min. The extract was centrifuged, and the supernatant was seperated to be used as test solution. The reference solution was prepared by dissolving 10 mg of borneol R and 15 mg of piperine R in 10 ml of methanol R. As the stationary phase, TLC silica gel F_{254} plate R (2–10 µm) was used, and the mobile phase consisted of *ethyl acetate* R and *cyclohexane* R 30:50 (*v/v*). Each solution was applied as bands of 10 mm, and the plate was developed over a path of 6 cm. After development, the plate was dried in air. The spots corresponding to the reference substances and the test solution were observed in the chromatograms under UV light, afterwards the plate was sprayed with anisaldehyde solution R and heated at 100°C for 5 min and examined in daylight [14].

Foreign Matter Examination

Herbal drugs were examined to ensure the absence of mold, insects, or other animal residues. Parts derived from the plant but not defined as herbal drugs, materials not originating from the plant, and substances of plant or mineral origin were considered foreign matter. During the test, a sample of 10 g, was weighed and spread in a thin layer. The foreign matter was examined with the naked eye. Any foreign substances were separated, weighed, and their percentage was calculated [14].

Loss on Drying

Loss on drying was determined according to the pharmacopoeia by drying 1 g of the powdered sample in an oven at 105°C for 2 hours [14].

Total Ash Determination

A crucible for each sample was heated to redness for 30 minutes, then cooled in a desiccator.

Subsequently, 1.00 g of powdered plant material was evenly distributed into the crucible. The sample was dried at 100–105°C for 1 hour and then incinerated in a muffle furnace at 600 ± 25°C until a constant weight was achieved [14].

Essential Oil Determination

Coarsely powdered herbal drug (25.0 g) was placed into a 1000 ml round-bottom flask along with 400 ml of water R, which served as the distillation liquid. Additionally, 0.5 ml of xylene R was added to a graduated tube. Distillation was carried out at a rate of 2-3 ml/min for 3 hours [14].

Liquid Chromatography Piperine Assay

The reference solutions and the test solution were prepared according to the methods given in the monograph [14].

The test solution was prepared by dispersing 0.250 g of powdered herbal drug in 40 ml of ethanol (96%) R. The mixture was sonicated for 20 minutes and then filtered. The contents of the flask and the filter were washed with 5 ml of ethanol (96%) R, and the filtrate and washings were combined. The volume was adjusted to 50.0 ml with the same solvent, and the solution was filtered through a membrane filter with a nominal pore size of 0.45 µm. Reference solution (a) was prepared by dissolving 15.0 mg of piperine CRS in ethanol (96%) R and diluting to 100.0 ml with the same solvent.

Reference solution (b) was prepared by dispersing 0.250 g of long pepper for system suitability HRS in 40 ml of ethanol (96%) R, followed by sonication for 20 minutes and filtration. The flask and filter were rinsed with 5 ml of ethanol (96%) R, and the filtrate and washings were combined and diluted to 50.0 ml with the same solvent. The solution was filtered through a membrane filter with a nominal pore size of 0.45 µm.

A system of Agilent 1260 series with DAD detector was employed for the analyses. The samples were injected in a volume of 10 µL and the detection was done at a wavelength of 343 nm. The column used had a length of 0.15 m and a diameter of 4.6 mm, with a stationary phase of C_{18} (5 µm). The flow rate was set at 1 ml/min. The mobile phase consisted of Water R as mobile phase A and Acetonitrile R as mobile phase B. Gradient elution used for the analyses is as follows: Between 0-5 minutes, the ratio of mobile phases A and B remained constant (50% - 50%). Between 5-20 minutes, the mobile phase A gradually decreased from 50% to 5%, while mobile phase B increased from 50% to 95%. Between 20-22 minutes, phase A was completely eliminated (0%), and phase B reached 100%.

The percentage of piperine content was calculated using the formula given in Equation 1:

$$Piperine\ \% = \frac{A1 \times m2 \times p}{A2 \times m1 \times 2} \qquad Equation\ 1$$

A_1 = area of the peak due to piperine in the chromatogram obtained with the test solution;
A_2 = area of the peak due to piperine in the chromatogram obtained with reference solution (a);
m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;
m_2 = mass of *piperine CRS* used to prepare reference solution (a), in grams;
P = percentage content of piperine in *piperine CRS*.

RESULT AND DISCUSSION

Organoleptic Control

According to the Turkish Pharmacopoeia 2017, Piperis longi fructus samples are measuring 10-25 mm in length (rarely exceeding 25 mm) and 3-5 mm in diameter [14]. Considering the identification criteria specified in the pharmacopoeia, five parts from each sample were examined. The results are presented in Table 1 for lengths, Table 2 for diameters, and Table 3 for the dimensions of the berry-like fruits. The results were recorded, and the average lengths were calculated to assess compliance with the pharmacopoeia.

The lengths, diameters, and berry-like fruit sizes of the samples were found to be compliant with the pharmacopoeia, except for Sample 7 and Sample 8, whose diameters did not meet the pharmacopoeial standards.

Table 1. Lengths of Piperis longi fructus in mm

Sample Code	Part 1	Part 2	Part 3	Part 4	Part 5	Mean	Compliance
Sample 1	30	29	39	19	18	27	Complies
Sample 2	26	31	30	34	23	28.8	Complies
Sample 3	36	23	25	24	28	27.2	Complies
Sample 4	18	45	25	24	20	26.4	Complies
Sample 5	20	24	25	23	28	24	Complies
Sample 6	36	32	25	26	20	27.8	Complies
Sample 7	38	34	37	30	27	33.2	Complies
Sample 8	37	40	44	25	27	34.6	Complies
Sample 9	40	28	21	25	20	26.8	Complies
Sample 10	41	38	44	37	40	40	Complies
Sample 11	32	39	19	25	25	28	Complies
Sample 12	36	27	32	35	30	32	Complies

Table 2. Diameters of Piperis longi fructus in mm

Sample Code	Part 1	Part 2	Part 3	Part 4	Part 5	Mean	Compliance
Sample 1	4	4	5	3	5	4.2	Complies
Sample 2	5	4	3	6	4	4.4	Complies
Sample 3	5	4	3	4	4	4	Complies
Sample 4	2	8	5	4	4	4.6	Complies
Sample 5	4	3	6	5	4	4.4	Complies
Sample 6	6	5	3	6	5	5	Complies
Sample 7	6	6	7	5	5	5.8	Not Complies
Sample 8	6	3	4	3	3	3.8	Not Complies
Sample 9	4	3	2	2	3	2.8	Complies
Sample 10	6	5	6	3	4	4.8	Complies
Sample 11	6	5	7	3	4	5	Complies
Sample 12	5	4	5	5	3	4.4	Complies

Table 3. Dimensions of berry-like fruits in mm

Sample Code	Part 1	Part 2	Part 3	Part 4	Part 5	Mean	Compliance
Sample 1	2	1	1	1	0.75	1.15	Complies
Sample 2	1	0.9	1	1	1	1	Complies
Sample 3	1	1,1	1	1	1	1.02	Complies
Sample 4	0.5	2	1	2	1	1,3	Complies
Sample 5	1	0.5	1.5	1	1	1	Complies
Sample 6	1	1	1.5	1	1	1.1	Complies
Sample 7	1	1	1	2	1	1.2	Complies
Sample 8	2	1	1	1	1	1.2	Complies
Sample 9	1	1	0.3	0.2	1	0.7	Complies
Sample 10	2	1	1	0.5	1	1.1	Complies
Sample 11	1	1.5	2	1	1	1.3	Complies
Sample 12	1.5	1.5	1	1.5	0.5	1.2	Complies

Microscopic Examination

The samples were powdered and examined under a microscope at 40x magnification using chloral hydrate R and Sartur R. The presence of the specified elements was observed in all samples. All samples were found to be compliant with the pharmacopoeia. The following microscopic elements were observed in the analyzed samples. The structures in the image correspond to a 50 µm scale. (Figure 1).

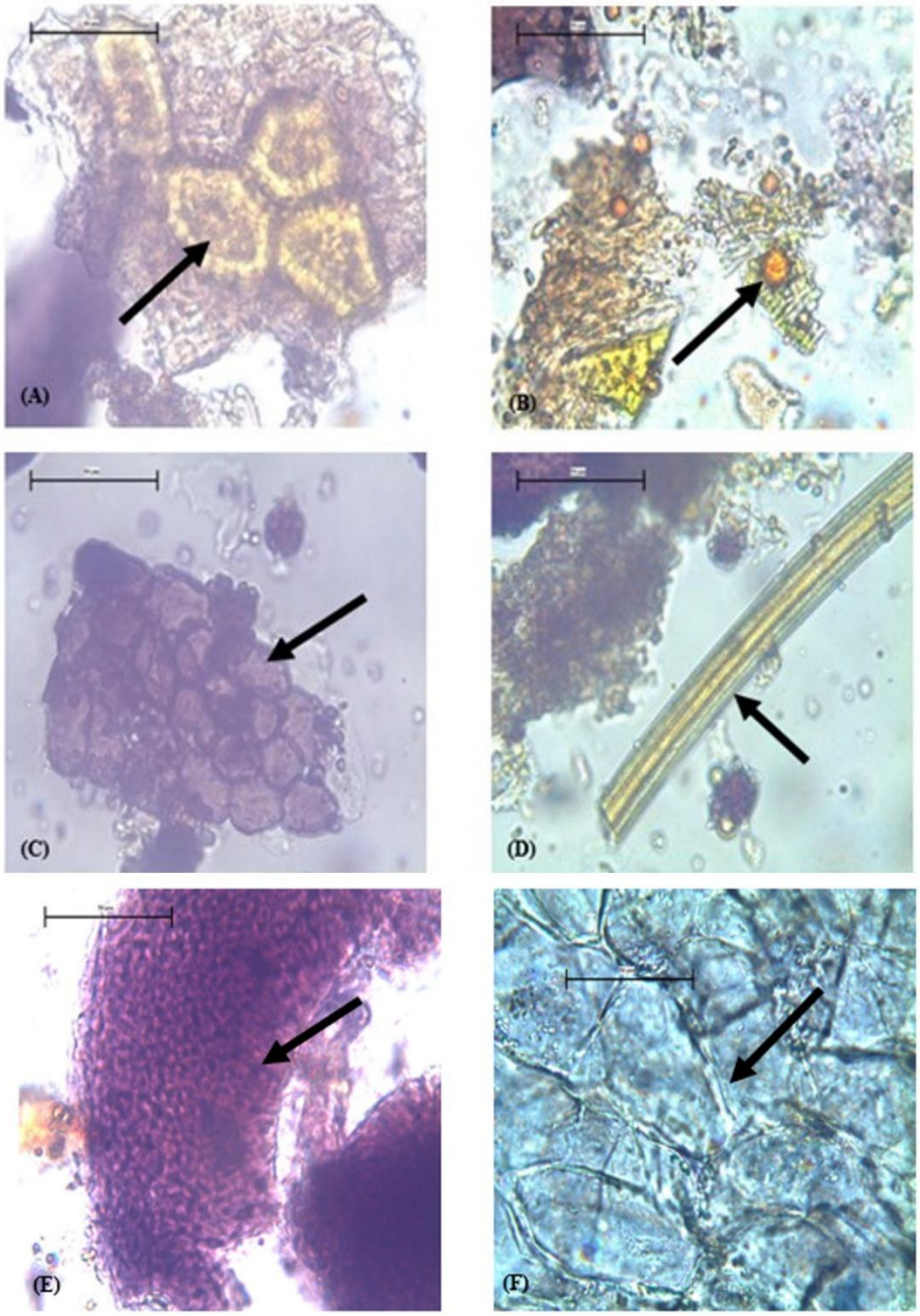


Figure 1. The microscopic elements observed in the analyzed samples (A) idioblast; (B) oil cell; (C) endocarp; (D) vessel; (E) starch cell; (F) parenchyma

Thin Layer Chromatography

According to the monograph, reference piperine reveals a single quenching zone and in the test

solution, a strong quenching zone appears at the same level as the reference piperine, confirming its presence. Additionally, several strong and faint quenching zones are observed at different positions. After treatment with anisaldehyde solution R, a yellowish-brown zone indicates the presence of borneol, and a green or brownish zone indicates the presence of piperine, in the reference solution. In the test solution, zones of similar color and position confirm the presence of both piperine and borneol. In addition, several zones in purple, violet, and purple-grey are observed.

The TLC chromatograms under UV 254 nm (Figure 2) and in daylight after reagent application (Figure 3) revealed the spots specified in the pharmacopoeia. Based on the analysis, the spots observed in the 12 test solutions matched those specified in the pharmacopoeia. Therefore, the samples comply with the standards of the Turkish Pharmacopoeia in terms of TLC analyses.

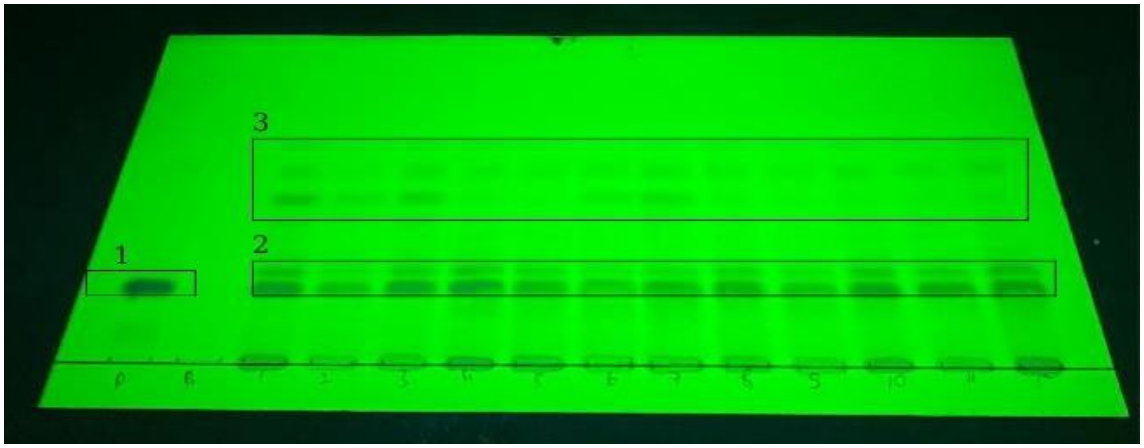


Figure 2. TLC chromatogram of reference and test solutions under UV light at 254 nm (1) piperine: a quenching zone; (2) a quenching zone (piperine); (3) a strong quenching zone

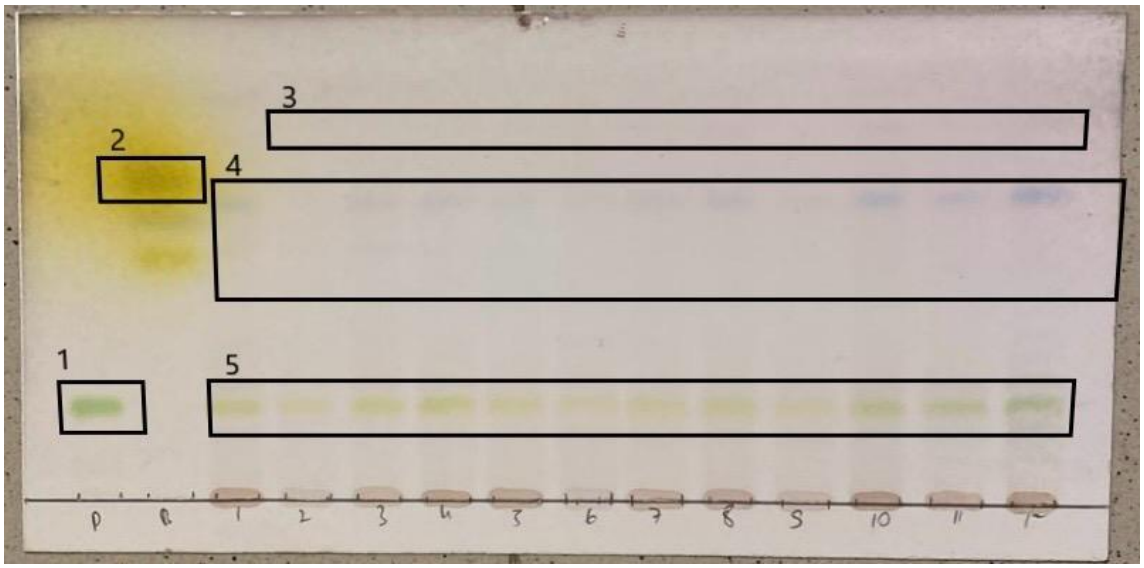


Figure 3. TLC chromatogram of reference and test solutions in daylight (1) piperine: a green or brownish zone; (2) borneol: a yellowish-brown zone; (3) a purple-grey zone; (4) a purple zone, violet zone, purple-grey zone; (5) a green or brownish zone (piperine)

Foreign Matter Analysis

The foreign matter ratios of the samples are presented in Table 4. According to the Turkish Pharmacopoeia 2017, the foreign matter content must not exceed 3.0%. All samples complied with the

pharmacopoeia, except for Sample 8 and Sample 9.

Table 4. Percentage of foreign matter in Piperis longi fructus

Sample Code	Weight	Foreign Matter	Percentage (%)	Compliance
Sample 1	14.5287	0.0272	%0.1858	Complies
Sample 2	37.6713	0.6845	%1.8170	Complies
Sample 3	11.8127	0.0277	%0.2344	Complies
Sample 4	13.9112	0.1176	%0.8453	Complies
Sample 5	12.9748	0.1310	%1.0096	Complies
Sample 6	18.7791	0.1613	%0.8589	Complies
Sample 7	11.1838	0.0172	%0.1537	Complies
Sample 8	48.7678	2.2723	%4.6594	Not Complies
Sample 9	18.0128	0.7635	%4.2386	Not Complies
Sample 10	23.2200	0.0499	%0.2149	Complies
Sample 11	14.7279	0.0861	%0.5846	Complies
Sample 12	17.4311	0.1054	%0.6046	Complies

Loss on Drying

According to the Turkish Pharmacopoeia, the loss on drying was checked and confirmed not to exceed 11%. Sample loss on drying was found to be in compliance with the pharmacopoeia. Table 5 shows the drying loss percentage results of Piperis longi fructus.

Table 5. Percentage loss on drying of Piperis longi fructus

Sample Code	Loss on Drying (%)	Compliance
Sample 1	7.1613	Complies
Sample 2	7.585	Complies
Sample 3	4.9452	Complies
Sample 4	7.1028	Complies
Sample 5	6.5441	Complies
Sample 6	5.652	Complies
Sample 7	8.8668	Complies
Sample 8	6.1222	Complies
Sample 9	7.2242	Complies
Sample 10	6.7389	Complies
Sample 11	5.806	Complies
Sample 12	1.7568	Complies

Total Ash Content Analysis

According to the Turkish Pharmacopoeia, the total ash content must not exceed 6.0%. The total ash content of the samples was found to be compliant with the pharmacopoeia, as shown in Table 6.

Table 6. Piperis longi fructus total ash amounts

Sample Code	Weight (g)	Total Ash (g)	Percentage (%)	Compliance
Sample 1	1.0440	0.0479	4.58812	Complies
Sample 2	1.0090	0.0387	3.83548	Complies
Sample 3	1.0030	0.0564	5.62313	Complies
Sample 4	1.0350	0.0546	5.27536	Complies
Sample 5	1.0140	0.0381	3.75740	Complies
Sample 6	1.0250	0.0376	3.66829	Complies
Sample 7	1.0040	0.0353	3.51594	Complies
Sample 8	1.0610	0.0559	5.26861	Complies
Sample 9	1.0170	0.0504	4.95575	Complies
Sample 10	1.0130	0.0472	4.65943	Complies
Sample 11	1.0070	0.0511	5.07448	Complies
Sample 12	1.0150	0.0533	5.25123	Complies

Essential Oil Content Analysis

According to the Turkish Pharmacopeia, the volatile oil content of Piperis longi fructus must be at least 6 ml/kg (anhydrous drug). At the end of distillation process, it was determined that the essential oil content of samples coded 1, 4, 7, 9, 10, 11, and 12 complied with the pharmacopoeia. The results are shown in Table 7.

Table 7. Piperis longi fructus essential oil contents

Sample Code	Weight(g)	Essential Oil	ml/kg	Compliance
Sample 1	25.333	0.225	8.8817	Complies
Sample 2	25.013	0.100	3.9979	Not Complies
Sample 3	25.034	0.150	5.9919	Not Complies
Sample 4	25.017	0.170	6.7954	Complies
Sample 5	25.015	0.150	5.9964	Not Complies
Sample 6	25.000	0.090	3.6000	Not Complies
Sample 7	25.088	0.190	7.5733	Complies
Sample 8	25.088	0.100	3.9860	Not Complies
Sample 9	25.171	0.190	7.5484	Complies
Sample 10	25.840	0.220	8.5139	Complies
Sample 11	25.055	0.270	10.7763	Complies
Sample 12	25.140	0.130	5.1710	Not Complies

Liquid Chromatography Piperine Assay

According to the pharmacopoeia, the piperine content should be at least 3% In Figure 4-6, the chromatograms of reference A, reference B and the chromotogram of one sample is given.

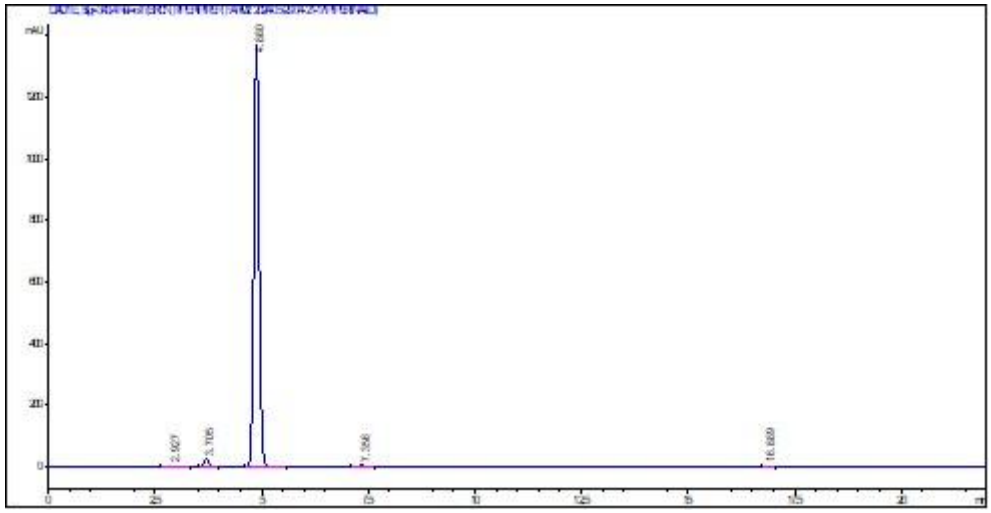


Figure 4. HPLC chromatogram of the reference solution A at 343 nm

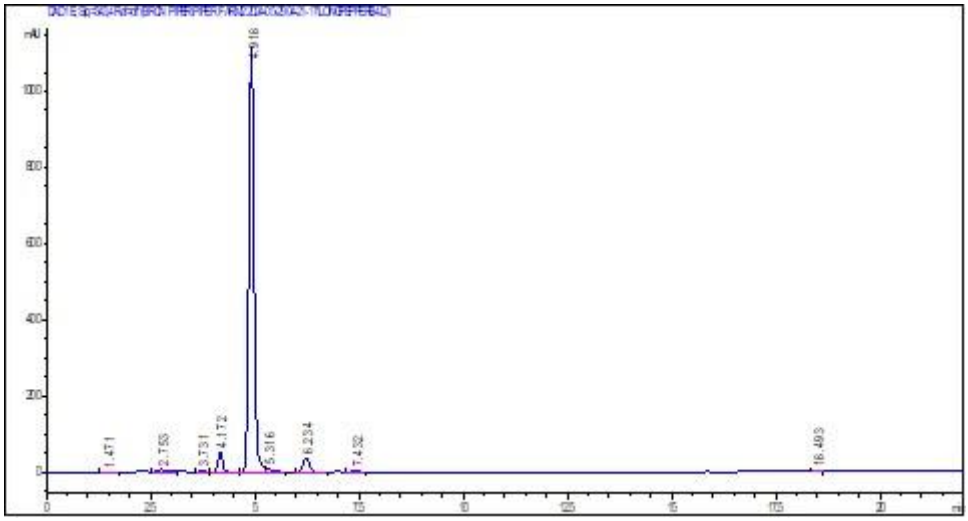


Figure 5. HPLC chromatogram of the reference solution B at 343 nm

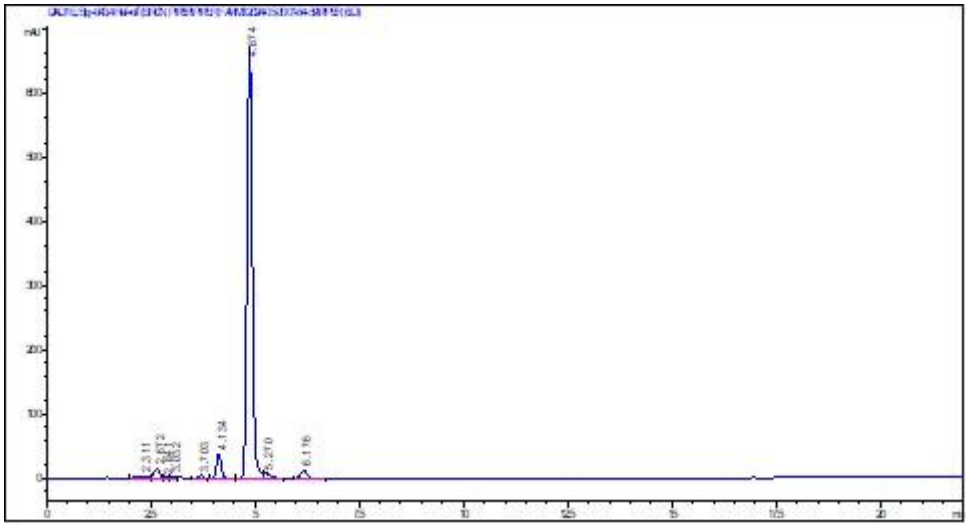


Figure 6. HPLC chromatogram of the sample 6

None of the samples were found to comply with the Turkish Pharmacopeia in terms of piperine content (Table 8).

Table 8. The piperin contents of the samples and reference solutions determined via liquid chromatography method

Sample	Peak Area	Piperine %	Pharmacopeial Compliance
Reference solution A	11833.3	-	-
Reference solution B	9707.4	-	-
Sample 1	6853.1	1.6505	Not Complies
Sample 2	6409.733	1.5437	Not Complies
Sample 3	5577.233	1.3432	Not Complies
Sample 4	8908.4	2.1455	Not Complies
Sample 5	11036.17	2.6580	Not Complies
Sample 6	5696.667	1.372	Not Complies
Sample 7	5911.233	1.4236	Not Complies
Sample 8	7160.9	1.7246	Not Complies
Sample 9	10559.13	2.543	Not Complies
Sample 10	7789.9	1.8761	Not Complies
Sample 11	10978.8	2.6441	Not Complies
Sample 12	9942.333	2.3945	Not Complies

Piper longum is considered a potential remedy due to its antitumor, anti-diabetic, hepatoprotective, anti-apoptotic, antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities [15]. Additionally, it has been shown that piperine enhances the bioavailability of various drugs such as sulfadiazine, tetracycline, streptomycin, rifampicin, pyrazinamide, ethambutol, and phenytoin. Considering its significant effect on increasing the bioavailability of drugs, there is potential for its use as an adjuvant with therapeutic drugs in chronic conditions, resulting in a reduced effective dose of drug intake, and thus minimizing subsequent adverse effects [16].

Pharmacopoeias are scientifically and legally authoritative reference sources published by national or regional authorities to establish standards and quality specifications for pharmaceutical products. Compliance with the pharmacopoeia is essential for determining the safety of medicinal plant usage.

This study is the first scientific research to comprehensively evaluate the compliance of commercially available Piperis longi fructus samples in Türkiye with the quality and purity criteria specified in the Turkish Pharmacopoeia 2017 [14]. The study was conducted on twelve different samples obtained from the market.

Based on the evaluations for identification; except for samples 7 and 8, the dimensional assessments of Piperis longi fructus were found to be compliant with the pharmacopoeia.

According to microscopic examinations, all samples contained the specified elements, confirming pharmacopoeial compliance.

As the result of TLC analyses; all the spots to be observed according to the monograph were consistent with the experiments conducted. Considering the TLC evaluation, the samples obtained from the market were found to be compliant with the pharmacopoeia.

The results of the tests conducted for the determination of loss on drying and ash content were in compliance with the pharmacopoeia.

In the foreign matter determination, Sample 8 and Sample 9 did not comply with the pharmacopoeia. This result indicates that these samples are inadequate in terms of pharmaceutical purity and quality. All other samples were in compliance with the foreign matter determination in the pharmacopoeia.

As for the volatile oil content; samples 2, 3, 5, 6, 8 and 12 did not comply with the pharmacopoeia. A recent study suggests that the lower than predicted levels of essential oils may be due to the loss of

these compounds under inappropriate drying and storage conditions due to their high volatility. It has also been shown that different drying methods alter the composition of these compounds, leading to different volatile profiles [17]. Similarly, a study on *P. nigrum* found that the amount and composition of volatile oil in samples changed with drying and storage conditions [18]. In conclusion, considering that drying and storage conditions can significantly affect both the amount and composition of essential oils, it is suggested that the low essential oil levels detected in the samples in our study may have been caused by these unsuitable conditions.

The liquid chromatography analyses revealed that none of the samples' piperine content comply with the pharmacopoeia. As emphasized in the current literature, the conjugated double bond system and the methylenedioxyphenyl group in the piperine molecule are susceptible to photochemical reactions when exposed to unfavorable environmental conditions such as light and temperature, causing the molecule to break down. This leads to a decrease in the stability and bioavailability of piperine [19]. Therefore, it is suggested that the low piperine levels detected in our study may be related to photodegradation due to inappropriate drying and storage conditions.

Although the piperine levels measured in the studied samples did not meet the pharmacopoeia standards, they were found to vary between 1.34-2.65%. The variability of piperine content in the samples obtained from different geographical regions reveals that the chemical composition of the herbal drug varies depending on the region it grows [20]. In piperine levels between samples can also be explained in this context.

When the current literature data are examined; it is reported that the majority of herbal materials offered for sale in the market do not meet the pharmacopoeia standards. For example, Barak et al. evaluated the compliance of 13 different clove oil samples available in the Turkish market with the European Pharmacopoeia (EP) 10.0 standard and the results revealed that none of the samples fully met all the quality criteria specified in the monograph [21]. Demir and Gürer investigated the compliance of six different *Zingiber officinale* (ginger) samples obtained from the market with the European Pharmacopoeia (EP) 10.0 standard. As a result of the examinations, it was reported that the products were largely in compliance with the pharmacopoeia except for quality parameters such as loss on drying and essential oil content [22].

In a previous study conducted by this research group, the compliance of cinnamon, clove, tea tree and neroli essential oils, which are widely used, with the Turkish Pharmacopoeia 2017 criteria was evaluated; it was determined that only two of the 25 samples analyzed met the pharmacopoeia standards in all quality parameters [23].

Similar to these findings, the Piperis longi fructus samples evaluated in the present study did not meet the quality standards defined in the Turkish Pharmacopoeia 2017, especially in terms of piperine content and essential oil content. This situation points to the decrease in the therapeutic effect of herbal drugs at the active ingredient level and the difficulties encountered in ensuring standardization. In conclusion, as seen in this study and other findings in the literatures, a significant number of herbal products offered for sale in the market do not comply with pharmacopoeia standards. Therefore, to ensure product quality and therapeutic safety, it is necessary to adopt production processes in accordance with the standards, to carry out post-harvest processes in a controlled manner, to ensure appropriate drying and storage conditions, to increase the frequency of inspections of producers and importers, and to perform pharmaceutical analyses systematically. In addition, institutionalization of pharmacopoeia compliance audits at national level to ensure quality assurance of herbal products is of great importance.

ACKNOWLEDGEMENTS

This study was supported by the TÜBİTAK 2209-A Research Project Support Program for Undergraduate Students.

AUTHOR CONTRIBUTIONS

Concept: M.K., B.E.; Design: M.K., B.E.; Control: M.K., B.E.; Sources: M.K., B.E.; Materials: F.Y., G.İ.D.; Data Collection and/or Processing: M.K., B.E., F.Y., G.İ.D.; Analysis and/or Interpretation: M.K., B.E., F.Y., G.İ.D; Literature Review: F.Y., G.İ.D.; Manuscript Writing: M.K.,

B.E., F.Y., G.İ.D.; Critical Review: M.K., B.E.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Acıbuca, V., Budak, D.B. (2018). Dünya'da ve Türkiye'de tıbbi ve aromatik bitkilerin yeri ve önemi. Çukurova Tarım ve Gıda Bilimleri Dergisi, 33(1), 37-44.
2. Durusoy, Ç., Ulusal B.G. (2007). Dermatolojide bitkisel tedavi-fitoterapi. Türk Dermatoloji Dergisi, 1, 47-50.
3. Şarışen, Ö., Çalışkan, D. (2005). Fitoterapi: Bitkilerle tedaviye dikkat (!). Sted, 14(8), 182-187.
4. World Health Organization. (2013). WHO traditional medicine strategy: 2014-2023. https://iris.who.int/server/api/. Accessed date: 01.05.2025.
5. Dehelean, C.A., Marcovici, I., Soica, C. et al. (2021). Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules, 26(4), 1109. [CrossRef]
6. Jaleel, J. (2006). MSc Thesis. Characterisation of long pepper (*Piper longum* L.) genotypes using morphological, anatomical and molecular markers, Kerala Agricultural University, Thrissur, India.
7. Babu, K., Divakaran, M., Ravindran, P. et al. (2006). Long pepper. In Handbook of Herbs and Spices, Elsevier, Amsterdam, 420-437. [CrossRef]
8. Kumar, S., Kamboj, J., Sharma, S. (2011). Overview for various aspects of the health benefits of *Piper longum* Linn. fruit. Journal of Acupuncture and Meridian Studies, 4(2), 134-140. [CrossRef]
9. Khushbu, C., Roshni, S., Anar, P. et al. (2011). Phytochemical and therapeutic potential of *Piper longum* Linn a review. International Journal of Research in Ayurveda and Pharmacy, 2(1), 157-161.
10. Zaveri, M., Khandhar, A., Patel, S. et al. (2010). Chemistry and pharmacology of *Piper longum* L. International Journal of Pharmaceutical Sciences Review and Research, 5(1), 67-76.
11. Manoj, P., Soniya, E., Banerjee, N. et al. (2004). Recent studies on well-known spice, *Piper longum* Linn. Natural Products Radiance, 3(4), 222-227.
12. Günşar, F. (2017). Karaciğer hastalıklarında tamamlayıcı (komplementer) tedavi. Güncel Gastroenteroloji, 21, 211-215. [CrossRef]
13. Atal, C.K., Zutshi, U., Rao P.G. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. Journal of Ethnopharmacology, 4(2), 229-232. [CrossRef]
14. Türk Farmakopesi 2017. (2018). Genel monograflar II, TC Sağlık Bakanlığı Yayın No: 1098, 1.
15. Srivastava, P. (2014). Therapeutic potential of *Piper longum* L. for disease management-a review. International Journal of Pharma Sciences, 4(4), 692-696.
16. Jeon, H.J., Kim, K., Kim, Y.D. et al. (2019). Naturally occurring Piper plant amides potential in agricultural and pharmaceutical industries: Perspectives of piperine and piperlongumine. Applied Biological Chemistry, 62(1), 63. [CrossRef]
17. Monacci, E., Sanmartin, C., Bianchi, A. et al. (2025). Chemical quality and characterization of essential oils in postharvest hop cv. cascade: Ventilated room temperature as a sustainable alternative to hot-stove and freeze-drying processes. Beverages, 11(2), 54. [CrossRef]
18. Orav, A., Stulova, I., Kailas, T. et al. (2004). Effect of storage on the essential oil composition of *Piper nigrum* L. fruits of different ripening states. Journal of Agricultural and Food Chemistry, 52(9), 2582-2586. [CrossRef]
19. Wu, N., Yin, X., Cui, C. et al. (2025). Structural modification, total synthesis, and biological activity of natural product piperine: A review. Journal of Functional Foods, 128, 106827. [CrossRef]
20. Hamrapurkar, P., Jadhav, K., Zine, S. (2011). Quantitative estimation of piperine in *Piper nigrum* and *Piper longum* using high performance thin layer chromatography. Journal of Applied Pharmaceutical Science(Issue), 117-120.
21. Barak, T.H., Gültekin, E., Şentürk, T.B. et al. (2024). Evaluation of commercial *Syzygium aromaticum* L. (clove) essential oil samples from market in accordance with the European Pharmacopoeia 10.0 criteria. Journal of Research in Pharmacy, 28(4), 1088-1098.
22. Demir, K., Gürer, Ç. (2024). Pharmacopoeia studies on *Zingiber officinale* Roscoe (ginger) samples

purchased from a pharmacy and herbalists in İzmir and İstanbul. Clinical and Experimental Health Sciences, 15(1), 117-123. [CrossRef]
23. Ergene, B., Şen, R.S. (2024). Evaluation of the quality of some widely used essential oils according to the specifications given in Turkish Pharmacopeia 2017. Journal of Research in Pharmacy, 28(1), 164-176.