Clinical and Experimental Health Sciences

Pharmacopoeia Studies on Zingiber Officinale Roscoe (Ginger) Samples Purchased from a Pharmacy and Herbalists in İzmir and İstanbul

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Received: May 12, 2024 **Accepted:** March 13, 2025

ABSTRACT

Objective: The aim of this study is to discover if the samples of *Zingiber officinale* were compatible with the criteria written in the European Pharmacopoeia, as quality control tests, which the analyses were done with the samples supplied from a pharmacy and the herbal stores. Also gathering knowledge from the scientifical literature of the drug's activities a contribution was made to the phytotherapy.

Methods: One pharmacy and five herbalist samples were examined and the quality control tests were done due to the monograph in the European Pharmacopoeia. According to the monograph macroscopic and microscopic assays, thin layer chromatography with methanolic extract, loss on drying, total ash and essential oil determination assays accomplished.

Results: With the macroscopic analysis and the microscopic analysis, the pharmacy sample were compatible with the criteria stated in Pharmacopoeia but one sample from herbal store had some unwanted and unidentified particles. The results of the loss on drying test which is repeated three times for each sample showed that the pharmacy sample and one from herbal store has more moisture than stated in the Pharmacopoeia. Also, total ash determination test was done three times and found that all the samples met the criteria stated in the Pharmacopoeia 7.0.

Conclusion: The results of the essential oil determination tests showed us that the sample obtained from pharmacy and three of the herbal stores were meeting the criteria but the EO's of the other samples were under the amount stated in the Pharmacopoeia.

Keyword: Antioxidant, European Pharmacopoeia, *Zingiber officinale* Roscoe, Zingiberis rhizoma

1. INTRODUCTION

Zingiber officinale Roscoe is not a naturally growing plant in Türkiye, it has only one name and known as "zencefil." The plant is a member of Zingiberaceae family. Its name comes from two different thoughts. First one is the name comes from the Sanskrit name "singabera" (srngaveram–srngam) meaning horn and (vera) meaning body as it looks like a deer's horns (1). And the second comes from an antique language, Dravidian (Tamil and Malayalam), a spice called "inchi/inji" and afterwards addition of the word "ver" that means tuber (2).

There were detected 115 compounds in *Z. officinale* when a gas chromatography mass spectrometry is done (3). Literature search of *Z. officinale* showed that the terpenic compounds are responsible for the main activity; oleoresins that are responsible for the smell of it; gingerols that gives the hot sense on tongue when consumed; and starch with a very high ratio of %60 (4,5). In addition to these, protein; minerals like calcium, potassium, phosphate and magnesium (6); vitamins like A, B3, B5 and B6; folic acid; waxes; lipids; lecitine (6,7); musilage and resin (8); carbonhydrate and proteolitic enzymes like zingibaine (9); and aminoacids like arginine (10).

The Zingiberis rhizoma has oleoresins with the ratio of %4.7-7.5 in which quarter compound of it consists of gingerdiol, zingerone, diterpenes, diarilheptanes and diarilheptanoides which are responsible with the sharp taste of plant; non-volatile phenilpropaoide compounds like gingerols and shogaols which are responsible with the characteristic sharp taste of plant (3,7,9).

Gingerols are responsible with the anti-oxidant, anti-inflammatory, anti-microbial and enzyme-regulation metabolism activities (4,11,12). They can transform to first shogaol and zingeron and then to paradol with the hydrogenation of shogaol (9).

The rhizomes which are preserved and stored for a while offer more active ingredients than fresh ones. This can be seen with shogaols. (13).

Essential oil has approximately 24 compounds like monoterpenic and sesquiterpenic hydrocarbons, alcohols

https//doi.org/*10.33808/clinexphealthsci.1482709*

and aldehyde compounds like neral and geraniale. The main compounds are camphene, β-fellandrene, 1,8-cineole, geraniale and neral. As monoterpenes there are compounds like limonene, myrsene, α – pinen, borneol, sitronellol, geraniol, geranyl acetate. Sesquiterpenes exist which takes the %30-70 of volatile oil like β-bisabolene, (-)-zingiberene, (+)-*ar*-curcumene, (-)-β-sesquifellandrene. Zingiberen amount was calculated %31.08 in the essential oil (3,14,15,16)

When we look to the fatty oil it has a ratio of %46 of saturated oils like palmitic acid and a ratio of %53 of unsaturated oils like oleic and linoleic acid (3).

Tannen compounds play the role of hemorrhoid and burn healing activity. Zinc, chromium and manganese are the heavy metal ingredients and it can be called with the safe levels of them and with the absence of lead and cadmium (17).

The plant is used for many different biological activities. Some of common aim of these uses are anti-emetic, carminative, anti-oxidant, anti-microbial as anti-viral, anti – bacterial and anti-fungal activities and anti-inflammatory as anti-ulcerative and anti-arthritis activities (18).

Today, drug treatment is preferred as the efficacy of synthetic drugs has been proven in the treatment of diseases and the exact treatment results can be achieved with appropriate dosage and treatment methods with these synthetic drugs. However, both herbal and animal drugs are also used for the treatment of diseases because of their activities proven by both traditional information and scientific data. Especially, because of the side effects and unwanted effects of synthetic medicines, herbal products have become popular nowadays. And ginger is also considered as a good supplement with many biological activities. Besides these information, great attention must be paid to the side effects especially related to teratogenicity. Apart from the side effects, the reliabilities of these herbal drugs are also important due to the possibility of mixing them with other herbal drugs both intentionally or unconsciously. For this purpose, ginger is selected as the subject which has ethnopharmacological data and has been the subject of many scientific research with the information that is coming from old times about the usage in the public medicine.

The aim of the study was achieving the information that if the herbal drugs which are sold in pharmacies and the herbal stores in Turkey fulfill the properties stated in the European Pharmacopoeia. For this reason, samples bought from Istanbul and Izmir herbal stores and bought from a pharmacy is used in the pharmacognostic analysis and quality control tests according to European Pharmacopoeia 7.0.

2. METHODS

2.1. Plant Materials

The plant material samples were bought from 5 herbalists that were randomly chosen that three of them were in Izmir and two of them were in Istanbul. Unfortunately, one sample was obtained from pharmacy because of the absence of another GMP packaged sample. All the samples were coded. (Pharmacy sample: E and the herbal store samples: T1, T2, T3, T4 and T5) All the samples except the pharmacy sample were powdered. Pharmacy sample was powdered manually by a cutting mill (Retsch SM100) in laboratory.

2.2. Macroscopic Analysis

Specifications that were stated in the European Pharmacopoeia 7.0 was morphologically examined with all the six plant material. Only the pharmacy sample was examined for shape, length, color and texture specifications as it was the only rhizome drug. After powdering the pharmacy sample all of the six samples were examined under the loope.

2.3. Microscopic Analyses

All the plant material must be in the powdered form before examination under microscope. Each sample was examined as preparations that were prepared by using chloralhydrate. Under chloralhydrate characteristic brown oleoresins, brown cork and groups of large, thin-walled, septate fibers; fragments that are containing vessels often accompanied by narrow, thin-walled cells containing brown pigment and amyliferous parenchyma.

2.4. Thin Layer Chromatography (TLC)

For the test solution 1g of each powdered plant material were dissolved in methanol (Merck 106009). For the reference solution 10 µL of citral (Sigma C83007) and 10 mg of resorcinol (Merck 107593) were dissolved in 10 mL methanol. The reference solution must be freshly prepared. Mobile phase was hexane/ether with the ratio of 40/60 (V/V). After a development of 15 cm path in an unsaturated tank and drying in air, 10 g/L solution of vanillin in sulfuric acid was sprayed as reagent. The silica gel plates were heated at 100-105 °C for 10 minutes and examined in daylight.

2.5. Loss on Drying

For this test gravimetrical method was chosen. Due to this path glass crucibles were made constant weight by keeping in the 105 °C drying oven and after by cooling in a desiccator. Empty crucibles were weighted and 4 grams of sample were weighted and put in each of them. Subsequent to drying in the 105 °C oven for 2 hours, each crucible was cooled in the desiccator and reweighed. For every sample this procedure was repeated 3 times, and the weight loss percentage was calculated.

2.6. Total Ash

Porcelain crucibles were kept 30 minutes at 105 °C in the heating oven and then kept in the desiccator to cool down and get to constant weight. 1 gram of each sample were put

into a crucible after crucibles were weighted empty. After drying at 100 – 105 °C for an hour, they were burned by slowly increasing temperature from in ashing furnace (Protherm PC442T, Protherm Furnaces, Ankara, Turkey) till 600 °C until white ashes were seen. Flaming was not permitted. Then crucibles were cooled to constant weight in desiccator and then weighed again to find the percentage of ash to 1 gram of sample.

2.7. Determination of Essential Oils

20 grams of each coarsely powdered fresh sample were weighted and put into 1000 mL round-bottomed flask. Then 10 drops of liquid paraffin and 500 mL water were added for distillation. 0.5 mL of xylene was added into the graduated tube. At the end of the distillation the volume of collected essential oils were measured on the graduated tube. Minimum 15mL/kg essential oil is expected.

3. RESULTS

3.1. Macroscopic Analysis

Pharmacy sample was bought in sealed packages as rhizome drug. Colour was between light brown to golden yellow. It was covered with the hard cork with evident, narrow, longitudinal and transverse ridges and occasional loose fibres. Inner surface yellowish – light brown parts were seen. After powdering in the laboratory when looked under the loope pharmacy sample, it showed light yellowish – brown colour. The pharmacy drug was shown in Figure 1. Herbal store samples were bought in powdered form. They also showed light yellowish – brown colour. But there were some unwanted and unidentified particles in samples which didn't belong to ginger. In conclusion pharmacy sample was more reliable than herbal store samples according to European Pharmacopeia criteria. For more detailed information microscopical analyses were done.

***Figure 1**. Pharmacy sample before powdering and after powdering*

3.2. Microscopic Analyses

Powdered samples were examined under microscope with the mounting solution chloral hydrate. There were seen large, thin walled and septate fibers; and vessels brown colored and narrow and thin-walled cells and with amyliferous parenchyma; brown to yellow-colored cells containing oleoresins; brown cork fragments. For only one herbal store sample (T3) the unwanted and unidentified particles which were seen on macroscopic analyses, also seen on microscopical analyses. Some specific microscopic images were taken and can be seen in Figure 2.

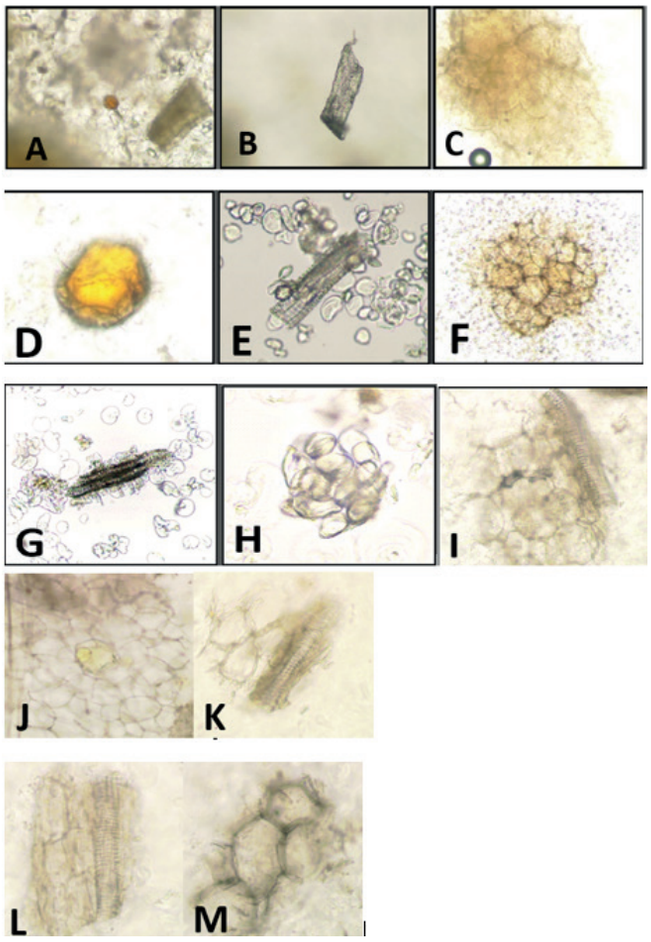

***Figure 2**. Microscopical images of the samples. Oleoresin (A), Reticulate vessel (B) and starch (C);*

T1 sample: Oleoresin (D), Reticulate vessel (E) and starch (F); T2 sample: Reticulate vessel (G) and starch (H); T3 sample: Reticulate vessel and starch (I); T4 sample: Oleoresin and starch (J) and Reticulate vessel and parenchyma (K); T5 sample: Reticulate vessel and parenchyma (L) and starch (M)

3.3. Thin Layer Chromatography

The results of the thin layer chromatography analyses were compatible with the criteria written in the European Pharmacopoeia 7.0 for all the samples.

The reference solution, that citral and resorcinol in methanol, gave us an intense red zone in the lower parts of the chromatogram because of resorcinol and two purple zones in the upper part because of citral.

The test solution, that sample in the methanol, applied chromatogram gave us two purple zones because of gingerols under the area of red zones because of the resorcinol in reference solution. In the middle parts there were two less intense purple zones caused by shogaols, between the two zones caused by resorcinol and citral that exists in the reference solution. Photos of two samples of the test results can be seen in Figure 3.

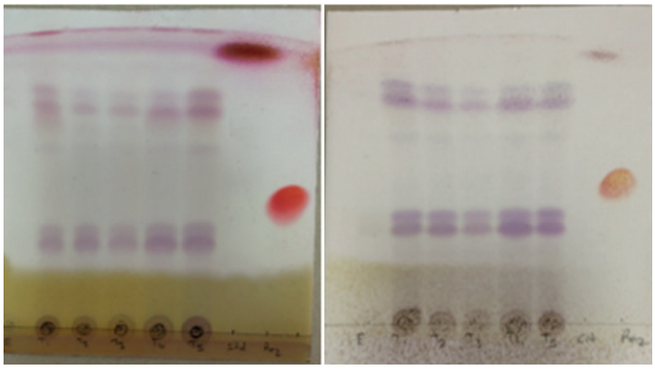

Figure 3. *TLC plates show zones of gingerols, resorcinol, shogaols and citral*

3.4. Loss on Drying and Total Ash

According to the monograph of *Zingiber officinale* in the European Pharmacopoeia 7.0 maximum 100 mL/kg of water is permitted from 20 grams of the powdered drug. Maximum %6 of total ash is indicated as criteria for each plant sample. The results of the tests can be seen on the Table 1. Results showed us that for all the samples total ash amounts remained under the criteria of maximum %6. For the water loss assay E and T2 samples were having more moisture than the criteria stated in the Pharmacopoeia. The other samples T1, T3, T4 and T5 remained under the maximum criteria.

Table 1. *Loss on drying test results*

Sample	Water Loss (g)	Total Ash (g)
E	0.42	0.04
T1	0.37	0.03
T2	0.43	0.04
T3	0.39	0.03
T4	0.39	0.04
T5	0.38	0.03

3.5. Essential Oil Test

Samples were tested for the amount stated in the pharmacopoeia of minimum 15 mL/kg essential oil. The essential oil accumulated in the graduated tube was measured. After measuring all the samples' essential oils, they were put to glass tubes to compare them relatively. The assay results can be seen on the table below. According to the results, pharmacy sample (E) and herbal store samples (T1, T2 and T5) had enough amount of essential oil as the criteria stated in the Pharmacopoeia. Herbal store samples T3 and T4 did not fulfill the same criteria.

Table 2. *Essential oil test results*

Sample	Result
T1	0.35 mL
T2	0.45 mL
T3	0.25 mL
T4	0.20 mL
T5	0.30 mL
E	0.40 mL

4. DISCUSSION

In this study, the *Zingiber officinale* drug samples which were bought from herbal stores and a pharmacy were controlled for the conformity of them to the specifications of monograph written in European Pharmacopoeia 7.0. Macroscopic and microscopic studies, thin layer chromatography, water test, total ash amount test and essential oil test on drug samples were done. The come outs were compared between each other for all the tests done for every test heading; and compared with the Pharmacopoeia data.

Zingiber officinale rhizomes which usually known as ginger have been used as a spice since ancient times. With scientifical researches on it the drug reveals a very great role in medicine with its active ingredients and so activities. And with the guide of these researches the drug serves a wide range of therapeutical activities like mostly known as carminative, antimicrobial and antioxidant effects.

The drug is being used since the Chinese and Indian traditional medicine. So, the outcomes of these knowledge since ancient times, today gives us many ways of usage of the drug. Anti-emetic activity and usage in the upper respiratory system infections are some examples for this situation (8, 14). As a traditional medicine in Turkey the drug is also used for antiemetic and antinausea effects.

The mature rhizomes, are harvested in the fall and have brownish-yellow color. It can be used as dried and then powdered drug or with making slices of the rhizome which is often used with honey together.

The main ingredients are active ingredients like gingiberol, gingerol, shogaol, citral, geranial and neral; curcuminoids and starch with the ratio of approximately half of it. The main aroma of the plant is because of the active ingredient gingiberol (19).

In human body free radicals that are generated throughout oxidation pathway, can cause temporary or permanent damage especially in the nervous system, reproductive system, and liver leading to significant problems. So many researches were done about the antioxidant activity of the drug. When calculated with the terms of quercetin it showed a very high activity (20). In another research, with

the higher dose of ginger, the MDA level which is related to the increasing free radical levels, decreased and the SOD and GSH levels which are the sign of the body defense, exhibited a significant increase. This antioxidant activity is maybe due to the high polyphenolic active ingredients of ginger that shogaol, 6-gingerol and 6-paradol (21). With its high antioxidant activity properties ginger is also a good antioxidant for reproductive system (22,23).

Highly alleviating vomiting and nausea as effective as vitamin B6 and having any teratogenic effect, make ginger more important for pregnant women (24). When compared with dimenhydrinate in a study, ginger extract showed similar effect with lower side effects, except one heartburn case in the study (25).

Although according to the British Herbal Compendium (BHC) usage in pregnancy related nausea is advised that ginger has very little side effects and this increases its reliability, the German Commission E and ESCOP approach cautiously and do not recommend its use in this kind of usage (26).

Studies made about post-operative patient care have shown that ginger can have anti-emetic activity as high as metoclopramide (27,28).

Besides these main activities ginger has activities like being an agent against migraine, obesity and diabetes, anti-thrombotic and hepatoprotective effects and protective effects against radioactivity. Regulating menstrual bleeding and angiogenesis are the other benefits of ginger.

The drug is registered in the European Pharmacopoeia, French Pharmacopoeia, British Pharmacopoeia, Swiss Pharmacopoeia, ESCOP, Commission E and WHO Monographs.

According to German Commission E the daily dosage must be between 2-4 grams (7). The high dose of the drug may cause gastrointestinal disturbances and burning, diarrhea, contact dermatitis, cardiac arrhythmia, central nervous system depression and allergic reactions related to immunoglobulin E through inhalation (29,30,31,32).

The results of the macroscopic and microscopic studies showed that the sample bought from pharmacy is suitable to the specifications of Pharmacopoeia and the herbal store samples (T1-T5) were also suitable to the criteria but they had some other unwanted and unidentified particles that must not be in the plant sample obtained from herbal stores.

The shogaols and the gingerols were seen within thin layer chromatography analysis which was shown in the Pharmacopoeia.

According to the results of the loss on drying test, pharmacy sample (E) and one sample of herbal stores (T2) were having higher moist loss values (0.42 and 0.43 g respectively) than the values stated in Pharmacopoeia. The other samples bought from herbal stores (T1, T3, T4 and T5) were having values between 0.37-0.39 g which were under the stated value which is 100 mL/kg and was 0.4 g for this study. All the samples met the criteria of Pharmacopoeia as they had %3-4 total ash.

The essential oils obtained from the samples were calculated in the range of 0.25-0.45 mL. The samples bought from herbal stores (T3 and T4) were under the criteria and the samples bought from pharmacy (E) and the herbal stores (T1, T2 and T5) were appropriate for the criteria while having values under 0.3 mL. According to the ecological conditions of a plant's production area and the production circumstances, plant's essential oil constituents and the yield changes. The other factors that affect the yield are harvesting date, the time passed since the plant material reaches laboratory where studied and the storage conditions. So, the differences of the studied samples' result values may be referred to these.

So, at the end of the study, it was observed that all the herbal store samples meet almost all the requirements of the European Pharmacopoeia 7.0. But, considering the production and packaging pathways and storage conditions, and taking into account that the use of herbal products under the supervision of a pharmacist would be more appropriate for patient health, it was concluded that the pharmacy drug would be more reliable. Drugs with medicinal properties should be made available to public health through pharmacies, in compliance with pharmacopeial standards and properly packaged.

5. CONCLUSION

This is the first study in Türkiye about *Zingiber officinale* plant material samples' compliance with the criteria written in the monograph in the European Pharmacopoeia 7.0.

Starting from the folk usage about the drug, the scientifically proven effects were collected so that a guide-like source about *Zingiber officinale* was aimed and prepared for the ones who want to use the drug for medicinal purpose or start a scientifical research.

Considering the scientifical studies are being continued about the Zingiberis rhizoma drug, hoping that *in-vivo* studies then will increase and with the *in-vitro* studies, more reliable knowledge will be known about the plant and the drugs of it.

Acknowledgement: *We would like to thank Istanbul University for permission to access academic literature.*
Funding: *This study was supported by Istanbul University Research Project Unit (BAPSIS). Project number: 23383*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study in this article was completed before the year 2020 (25.12.2018), and since ethical committee approval was not an obligation at that time, there is no ethical committee approval.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: KD, ÇGÜ
Design of the study: KD

Acquisition of data for the study: KD, ÇGÜ
Analysis of data for the study: KD
Interpretation of data for the study: KD
Drafting the manuscript: KD, ÇGÜ
Revising it critically for important intellectual content: KD, ÇGÜ
Final approval of the version to be published: KD

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How to cite this article: Demir K, Gürer ÇÜ. Pharmacopoeia studies on Zingiber officinale Roscoe (ginger) samples purchased from a pharmacy and herbalists in İzmir and İstanbul. Clin Exp Health Sci 2025; 15: 117-123. https//doi.org/10.33808/clinexphealthsci.1482709