

Pharmacopoeia Analysis of *Citrus aurantium* L. Ssp. *Amara* engl. and its fixed oil content

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ABSTRACT

Objective: Our aim was to take attention to *Citrus aurantium* L. ssp. *amara* Engl. (Rutaceae), which grows widely along the Mediterranean in Turkey where it has very limited medicinal usage. The bitter orange fruits/peels were analysed to find out if they are suitable to the standards in European Pharmacopoeia 7.0. The peel and fixed oil were investigated to figure out the oil quality to apply in medicinal and cosmeceutical preparations. **Material/Methods:** *C. aurantium* were collected from different regions of Turkey, various fruit parts and their extracts were evaluated according to the Pharmacopoeia. The essential and fixed oils were obtained from their fruits and seeds, respectively. Seeds were crushed and fixed oil was obtained by cold squeezing. Essential oil yield, index analysis of fixed oil and drug analysis were conducted in compliance with the procedures. **Results and Discussion:** Evaluation of the peel was found appropriate to the Pharmacopoeia. Aydin, Iskenderun and Mersin regions have been suitable to cultivate valuable medicinally used product. Although *C. aurantium* has commercial and medicinal value around the world, it has not significant utilization in Turkey. Therefore this study could help recognizing its health benefits. Absolutely, phytochemical content should be taken into account before medicinal using.

Key words: Bitter orange, *Citrus Aurantium*, Pharmacopoeia analysis.

INTRODUCTION

Citrus aurantium L. ssp. *amara* Engl. known as bitter orange, Seville orange, sour orange (synonym. *C. aurantium* L. subsp. *aurantium*) belongs to Rutaceae family. It grows naturally in Northern India (Indian, Khasia Hills in Assam, and Central Provinces), Southeast Asia along with Mediterranean, temperate regions of America.¹ It has very limited medical usage, mostly used to make drinks and liquor in all over the world, although the peel is used to make jam, no commercial value in Turkey whatsoever.² The European Pharmacopoeia has description about *C. aurantium* flower, fruit epicarp-mesocarp and fruit tincture.³ The plant's fruit is 2.5-4.5 in width and 4-8 cm in length. Its surface is reddish-yellow or brown, the other surface grayish red-yellow. It has an aromatic odor and bitter tasting. The fragrance of the fruit

is mostly due to the volatile oils. The whole fruit contains flavonoids, terpenes, carotenoids and coumarins apart from the main constituent volatile oils.^{4,5} *p*-Synephrine from biogenic amines is important in terms of toxicity/safety.⁶ Aim of this study, is to take attention to bitter orange in Turkey. The peel, and were analyzed for its suitability to the European Pharmacopoeia. The fixed oil from the seeds also was analyzed in order to figure out the oil quality to apply in medicinal and cosmeceutical usage.

MATERIAL AND METHODS

Plant materials

Fruits of bitter orange were collected from Adana, Antalya, Izmir and Mersin districts in Turkey. The peels of the fruits were dried in the shade.

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Pharmacopoeia analysis of the fruit epicarps and mesocarps

Experiments were conducted according to European Pharmacopoeia 7.0.³

Determination of total ash of the peel

Weighed and powdered materials were placed in a tared crucibles. Then, they have been placed in a muffle furnace at 600 ± 25 °C (about 2.5 hours) and they were burned. All the ash content was calculated as grams.³

Determination of water content of the peel

The amount of water was calculated as described in the pharmacopoeia.³

Determination of extractable matter

Each powdered plant material (2 g) was extracted with water:ethanol (3 mL:7 mL). After filtration, the filtrate was evaporated to dryness in a water bath and dried in an oven at 100-105 °C for 3 hours.³

Volatile oil assay

The amount of essential oils was calculated through the apparatus specified in pharmacopoeia for each sample.³

Microscopic analysis of the fruit

Powdered light brown materials were examined under microscope using chloral hydrate R.³

Obtaining the fixed oil from the seed

The fixed oil was obtained by cold squeezing (patent number: 2014/12733—2014-GE-46339). This oil is reddish-yellow color. Fatty acids in the fixed oil were analyzed with Gas chromatography (GC) by using flame ionization detector.⁷ For determination of the tocopherols, high-performance liquid chromatography-fluorescence detection (HPLC-FLD) was utilized.⁸

Pharmacopoeia analysis of the fatty oils

Acid value

1 mL of the substance to be examined was dissolved in 5 mL mixture of equal volumes of ethanol and ether. Phenolphthalein solution has been used as an indicator.

The mixture was titrated with 0.1 N alcoholic potassium hydroxide (KOH) until the pink color persists for at least 15 s.³

Saponification value

2 mL of substance to be examined was placed in a reflux condenser. 25 mL of 0.5 N alcoholic potassium hydroxide was added. It was heated under reflux. It was titrated immediately with 0.5 N hydrochloric acid in the presence of phenolphthalein. A blank test was carried out under the same conditions.³

Ester value

It is calculated from the saponification value and acid value.³

$$\text{Ester value} = \text{Saponification value} - \text{acid value}$$

Iodine value

The oil was dissolved in carbon tetrachloride and potassium iodide solution. Covered flask was kept in the dark for 30 min. Potassium iodide and dissolved starch solution were added to the flask, then titrated with 0.1 N sodium thiosulfate until the yellow color discharged. A blank test was carried out under the same conditions.³

Peroxide value

The oil was dissolved in glacial acetic acid: chloroform (18:12 mL) mixture and, 0.5 mL of saturated potassium iodide was added. Then 30 mL of water was added, the mix was titrated with 0.01 N sodium thiosulfate. Starch solution is added as indicator. And a blank test is carried out under the same conditions.³

RESULTS

Pharmacopoeia analysis on epicarp and mesocarp

Quantity of total ash, volatile oil, water and extractable matter were given Table1.

Microscopic analysis

Microscopic elements of fruit were given Figure 1.



Figure 1: Microscopic examination of samples. a) Epicarp with thick cuticle, collenchymatous hypodermis, b) Fragments of lysisigenous oil glands c) Epicarp with anomocytic stoma-in surface view, d) Fragment of mesocarp containing crystal of calcium oxalate, Mesocarp.

Table 1: Results of the pharmacopoeia analysis of the *C. aurantium* fruit peel samples

Collection site	Total ash (g/g)	Volatile oil (mL/g)	Extractable matter (mg)	Water content (mL/g)
ADANA	3,14 ± 0,07	0,45 ± 0,24	165,43 ± 19,83	8,13 ± 0,53
ADANA- Gülen	3,63 ± 0,11	0,58 ± 0,47	118,13 ± 36,44	5,96 ± 0,76
ANTALYA	4,41 ± 0,15	0,26 ± 0,14	157,07 ± 22,03	7,11 ± 0,16
AYDIN	3,92 ± 0,08	0,75 ± 0,50	155,00 ± 15,63	9,99 ± 0,01
HATAY- Dörtyol	3,20 ± 0,29	0,91 ± 0,20	205,27 ± 49,65	4,62 ± 3,01
İSKENDERUN	3,32 ± 0,20	1,04 ± 0,32	265,40 ± 34,87	7,49 ± 0,72
İZMİR	1,96 ± 0,16	1,09 ± 0,10	119,63 ± 47,61	9,22 ± 1,05
MERSİN	2,96 ± 0,22	0,95 ± 0,04	233,93 ± 23,90	6,99 ± 0,69
MERSİN- Alata	5,13 ± 0,07	0,64 ± 0,21	184,93 ± 32,09	6,00 ± 0,71
MERSİN- Pınar	4,95 ± 0,33	0,98 ± 0,08	255,00 ± 17,42	5,48 ± 2,14
MERSİN- Silifke	3,81 ± 0,32	0,91 ± 0,17	288,33 ± 16,08	8,24 ± 1,05
OSMANİYE	3,35 ± 0,24	0,91 ± 0,17	175,67 ± 17,24	6,61 ± 2,30

Table 2: Seed oil index values

Parameters	Seed oil index
Acid value	4,05 ± 0,22 KOH mg/1g
Saponification value	251,28 ± 2,80 KOH mg/1g
Ester value	247,24 ± 2,58 KOH mg/1g
Iodine value	31,28 ± 1,73 g/g
Peroxide value	0,50 ± 0,03 milliequivalents/kg

Table 3: Quantity of fatty acids

Fatty acid	Quantity (%)
Linoleic acid	33,02
Oleic acid	26,50
Palmitic acid	23,86
Linolenic acid	7,92
Stearic acid	7,11

Table 4: Quantity of tocopherols

Tocopherols	Quantity (mg/kg)
Alpha tocopherol	87 ± 4,736
Gamma tocopherol	30 ± 1,32

Pharmacopoeia analysis on fatty oils

Seed oil index was given Table 2.

Quantity of fatty acids and tocopherols

Quantity of fatty acids and tocopherols were given Table 3 and Table 4.

DISCUSSION

According to the results of peels, Aydın, Iskenderun and Mersin districts were suitable to cultivate desirable medicinal product. Peels of fruits gathered from these regions were appropriate to pharmacopoeia standard. Saponification, acid and ester index are very important to detect purity/quality control of drugs that carry volatile and fixed oils. The saponification index is a characteristic for each oil and is a parameter in determining the adulteration. Also, it gives information about fatty acids. The saponification index value of the bitter orange fixed oil was higher compared with olive oil. Acid value was directly proportional to saponification value. Another important criterion is iodine value which is degree of unsaturation. The presence of unsaturated fatty acid component in oil is essential for cardiovascular health. Oleic, linoleic and linolenic acids are unsaturated acids. Whereas oleic acid is lower, palmitic, linoleic and linolenic acid are more in bitter orange oil than olive oil. Iodine value is lower than compared olive oil's.⁹ Also, peroxide value of bitter orange oil was quite low. We can say that oxidation did not occur in bitter orange oil and this oil did not deteriorate. Different isomeric forms of tocopherols conduce fundamentally to the health improvement.¹⁰⁻¹² Tocopherols which are a precursor of vitamin E, especially gamma tocopherol, relatively large quantity in bitter orange oil. When bitter orange oil compared with olive oil that is important nutritional and medicinal oil in terms of fat's index values, we couldn't reach the result of using bitter orange oil instead of olive oil. Bitter orange oil is promising the future in terms of its fatty acids even if bitter orange oil is not an alternative to

olive oil. Besides, phytochemical content must be taken into account before using.

CONCLUSION

Herbal medicine sector should focus on bitter orange to produce medicinal and dietary products because of its rich phytochemical content. Although this plant is medicinally used widely around the world, it has not widespread utilizing in Turkey. Through this study, its benefits will be recognized and will be allowed to be consumed by Turkish people.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

ABBREVIATION USED

G: gram; GC: Gas chromatography; HPLC: High-performance liquid chromatography; HPLC-FLD: High-performance liquid chromatography-fluorescence detection; kg: kilogram; KOH: potassium hydroxide; Mg: milligram; Min : minute; mL: milliliter; N: normality

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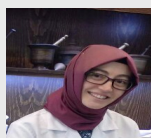
PICTORIAL ABSTRACT



SUMMARY

- Pharmacopoeia analysis was conducted on bitter orange only grown in Turkey for the first time.
- The fixed oil of bitter orange was obtained by cold squeezing.
- Fatty acids and tocopherols in the fixed oil were determined chromatographic methods.
- Results of experiments were compared to olive oil which is very valuable for health.
- By this study, bitter orange's benefits will be recognized and will be allowed to be consumed by Turkish people.

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