Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Oleuropein amounts of olive leaves from different regions of Northern Cyprus

Hadeel Homssi, Muberra Kosar*

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, T.R. North Cyprus, Mersin 10 Turkey.

Abstract

Olive (*Olea europea* L.) trees are widely grown in Mediterranean region and leaves are used as a traditional medicine. Oleuropein is the main active compound for the olive leaves. Olive leaves have potentially beneficial effects on certain health conditions, including antimicrobial and antioxidant and other biological activities. These beneficial effects may be due to the antioxidant components of olive leaves, especially oleuropein. Oleuropein containing extracts are used in many supplements for different pharmacological activities. In this study, oleuropein amounts of olive leaves from seven different localities of North Cyprus were investigated by HPLC. Olive leaves were extracted by methanol according to European Pharmacopoeia's method. Oleuropein amounts of the extracts were calculated by reversed phase HPLC with PDA. Buyukkonuk and Guzelyurt samples contain the highest amount of oleuropein, followed by Tatlisu, Dipkarpaz, Lapta, Zeytinlik and Lefke.

Keywords

HPLC, North Cyprus, *Olea europea*, oleuropein.

Article History

Submitted: 30 October 2019 Accepted: 18 November 2019 Published Online: 30 December 2019

Article Info

***Corresponding author:** Muberra Kosar, email: muberra.kosar@emu.edu.tr

Research Article:

Volume: 2 Issue: 2 December 2019 Pages: 68-74

INTRODUCTION

Medicinal plants play a vital role in healthcare and have been widely used as main treatment for approximately all ailments in ancient times (Anza *et al.*, 2017). Chemical drug discovery was a revolution in the era of medicine. However, increase in the rater of drug resistance and presence of undesirable side effects led scientists to investigate safer alternatives to synthetic medications. Therefore, the prevalence of phytochemicals such as phenolic compounds, flavonoids and tannins, to which the likehood of resistance development is much lower, has been increasing (Qin and Hou 2017).

The Mediterranean diet is a modern nutritional concept that is useful to enhance life quality. The idea was based on the premise that animal fats were predisposing for heart diseases whereas unsaturated plant fats or oils promoted good health. By pressing (squeezing) or centrifuging the crushed fruit and the seed, olive oil can be obtained. All oils extracted from olives are sorted as Virgin Olive Oils and the highest quality Virgin Olive Oils are called Extra Virgin Olive Oil. This class contains high amounts of monounsaturated fatty acid (60 to 80% oleic acid), medium amounts of polyunsaturated fatty acid (4 to 20%) and small amounts of polyphenols, tocopherols, sterols and many aromatic compounds.

O. europaea subsp. *europaea* L. has also many other auxiliary uses. The olive fruits and leaves falling from mature trees provide animals a source for food. The remnants from the process like the pomace are used as plant fertilizer, compost, and fuel. In many countries, olive trees are planted extensively as decoration or for shade. Because the tree timber is durable, it is used for furniture, as kitchen equipment and ornamental items. It can be used as a windbreak for the lighter fruit or plants or flowers that may be affected by wind and pulled out of soil like orchid family flowers. Olives have a pretty famous and special history and are symbolic. The branch of olive was and still a symbol of grace, victory, peace and blessing. The leafy branches of the olive tree were ritually offered to gods and significant figures as immolation of purity and benediction. They were also offered to winners of competitions or victors of wars. Nowadays, olives have still been used in religious rituals and curative sessions. Olive trees are importantly mentioned in Romanian and Greek culture as well as in Holy books as in Torah, Bible and the Holy Quran (Kapellakis *et al.*, 2008; Talhaoui *et al.*, 2015).

The aim of this study is to investigate oleuropein amounts within the different region in Northern Cyprus. Leaf samples were collected from 7 different places in Northern Cyprus (Tatlisu, Buyukkonuk, Dipkarpaz, Zeytinlik, Lapta, Guzelyurt and Lefke) and oleuropein amounts were calculated using reverse phase HPLC.

MATERIALS AND METHODS

Plant materials

Olive leaves were collected from 7 different regions (Tatlisu, Buyukkonuk, Dipkarpaz, Zeytinlik, Lapta, Guzelyurt and Lefke) in North Cyprus during the first week of March 2018 which are in order. 500 g of olive leaves were gathered from every area and the leaves were detached from the stems. The leaves were left to dry at room temperature for one week. Then leaves were crushed and kept in dry storage until extraction.

Preparation of the extracts

The extraction was carried out with dried and powdered leaves according to the European Pharmacopoeia (2014). 1 g of olive leaves extracted with 50 ml methanol was sonicated at 60 ºC for 30 minutes. The mixture was left to stand and cool down. Afterwards, it was filtered and washed with methanol till 100 ml. The extract was stored in the fridge. Then the extract was diluted with distilled water 1/10 dilution by adding 100 µl of extract to 900 µl water. All samples were extracted twice at same conditions.

Oleuropein analysis by HPLC

Reversed phase HPLC method was performed for oleuropein analysis using an isocratic elution. Oleuropein was eluted from C18 column (150 x 0.46 mm, 5 □m) using methanol: water: acetic acid (70:30:1) as a mobile phase. Flow rate was 1 mL/min and injection volume was 20 µL. Oleuropein was detected by PDA detector at 240 and 280 nm and the retention time of oleuropein was 5.4 minutes. Oleuropein standards were prepared at 1 mg/mL concentration for stock solution and then five dilutions (0.15, 0.1, 0.08, 0.06 and 0.04 mg/ml) were prepared for calibration curve. All standards and extracts were injected 3 times and mean values and standard deviations were calculated.

RESULTS AND DISCUSSION

Olive leaves were collected from seven different locations from North Cyprus (Table 1). Oleuropein was identified as major compound in the leaf extract of *Olea europea* by authentic standard oleuropein. The UV spectrum is given in Figure 1 and the calibration curves were shown in Figure 2 for both wavelengths 240 and 280 nm.

Table 1: Oleuropein amounts in *Olea europaea* from different localities of North Cyprus.

Sample Name	Locality	Amount of oleuropein (%, in plant) 240 nm	Amount of oleuropein (%, in plant) 280 nm
Sample 1	Tatlisu	**0.079 ± 0.003***	**0.080 ± 0.004**
Sample 2	Buyukkonuk	**0.088 ± 0.006**	**0.087 ± 0.006**
Sample 3	Dipkarpaz	0.072 ± 0.006	0.072 ± 0.007
Sample 4	Zeytinlik	0.057 ± 0.007	0.057 ± 0.007
Sample 5	Lapta	0.065 ± 0.003	0.065 ± 0.004
Sample 6	Guzelyurt	**0.086 ± 0.004**	**0.087 ± 0.005**
Sample 7	Lefke	0.046 ± 0.004	0.046 ± 0.004

*mean ± SD (n = 6)

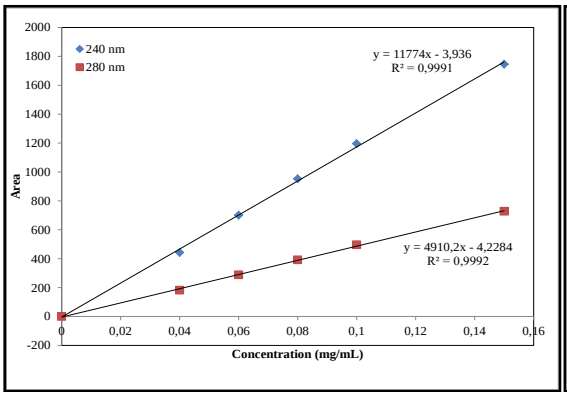

Figure 1: UV spectrum of *oleuropein*.

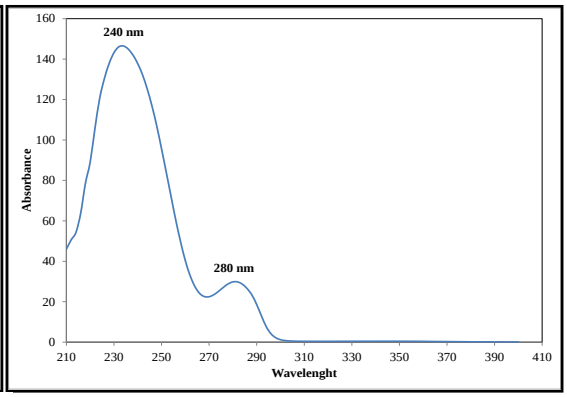

Figure 2: Calibration curves for oleuropein at 240 nm and 280 nm.

All samples were injected 3 times and at mean values and standard deviations are given in Table 1. The HPLC chromatograms of standard oleuropein and all extracts are given in Figure 3 and 4, respectively.

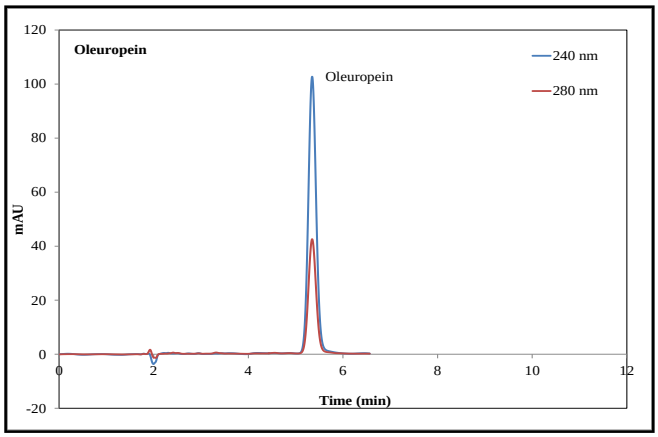

Figure 3: HPLC chromatogram of oleuropein.

After HPLC analysis of oleuropein within the extracts, areas of oleuropein were collected at 240 and 280 nm. The 240 nm is registered in European Pharmacopoeia and 280 nm in the specific Λmax from UV spectrum of oleuropein. The results for both wavelengths are given in Table 1.

Our results were accurate and show slight differences among the different localities. According to the results, Buyukkonuk, Guzelyurt and Tatlisu samples contained the highest amount of oleuropein but Lefke sample had less. The order of amounts of oleuropein within the extracts was as Buyukkonuk > Guzelyurt > Tatlisu > Dipkarpaz > Lapta > Zeytinlik > Lefke.

In Spain, olive leaves were analysed and different oleuropein derivatives and glycosides were detected between 0.028-0.329 % in dry mass (Talhaoui *et al.*, 2016). Oleuropein amount within the *O. europea* was found in wide range (0.03-2.16 %) according to the location of the raw materials (Vinha *et al.*, 2005).

According to the literatures (Talhaoui *et al.*, 2016; Hayes *et al.*, 2011; Czerwińska *et al.*, 2016), the results were correlated with the other Mediterranean countries.

In conclusion, oleuropein is considered as one of the most crucial secoterpenic compounds that have many biological benefits. Olive leaves were gathered from different locations in North Cyprus, and they were dried and then extracted with methanol, and injected to HPLC for qualification and quantification of the oleuropein within the samples. According to the results, oleuropein was found to be major phenolic compound within all olive leaves collected from different locations from North Cyprus. The order of the amount of oleuropein was found as Buyukkonuk > Guzelyurt > Tatlisu > Dipkarpaz > Lapta > Zeytinlik > Lefke.

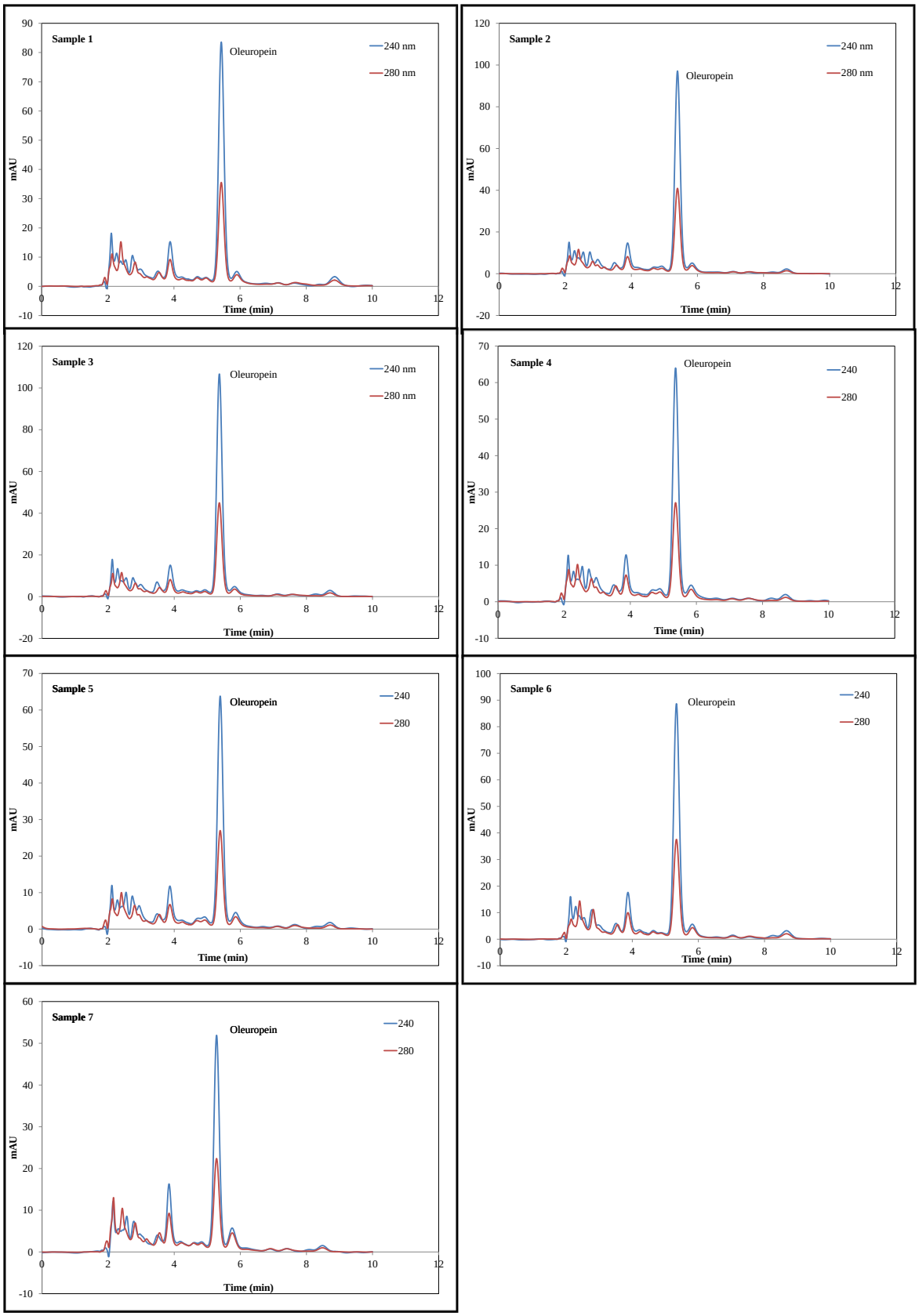

Figure 4: HPLC chromatogram of all samples.

REFERENCES

Anza M, Bibiso M, Alemayehu B, Desalegn E (2017). Phytochemical analysis, in vitro antioxidant and antibacterial activities of root extracts of *Carduus macracanthus*. *J Coast Life Med* **5**(11): 486-491.

Czerwińska ME, Duszak K, Parzonko A, Kiss AK (2016). Chemical composition and UV a-protecting activity of extracts from *Ligustrum vulgare* and *Olea europaea* leaves. *Acta Biol Cracoviensia Series Botanica* **58**(2): 45–55.

European Pharmacopoeia 8th edition, p. 1337, 2014.

Hayes JE, Allen P, Brunton N, O'Grady MN, Kerry JP (2011). Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. *Food Chem* **126(**3): 948-955.

Kapellakis IE, Tsagarakis KP, Crowther JC (2008). Olive oil history, production and by-product management. *Rev Environmental Sci and Bio/Technol* **7**(1**)**: 1-26.

Qin S, Hou D (2017). The Biofunctions of Phytochemicals and Their Applications in Farm Animals: The Nrf 2/Keap1 System as a Target. *Engineering* **3**: 738–752.

Talhaoui N, Taamalli A, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A (2015). Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. *Food Res Inter* **77**(2): 92-108.

Talhaoui N, Vezza T, Gómez-Caravaca AM, Fernández-Gutiérrez A, Gálvez J, Segura-Carretero A (2016). Phenolic compounds and in vitro immunomodulatory properties of three Andalusian olive leaf extracts. *J Functional Foods* **22**:270–277.

Vinha AF, Ferreres F, Silva BM, Valentão P, Gonçalves A, Pereira JA, Oliveira MB, Seabra RM, Andrade PB (2005). Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. *Food Chem* **89**(4): 561-568.