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Phenolic Content and *In-vitro* Antioxidant Activity of *Olea europaea* L. subs. *oleaster* Leaves by Supercritical CO_2 Extraction

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ÖZ

Bu çalışmada Akdeniz Bölgesi, Mersin ili Tarsus ilçelerinde doğal olarak yetişen ekonomik olarak önemli bir potansiyeli olan *Olea europaea* L. subs. *oleast*er (delice zeytin) çeşidine ait zeytin yapraklarının, süperkritik CO_2 ekstraksiyon yöntemi (SCFE) ile 150 bar ve 300 bar basınç altında elde edilen ekstraktlarının (sırasıyla OE1 ve OE2) total fenolik (TPC) ve total flavonoid (TFC) içeriklerinin ve in-vitro antioksidan aktivitelerinin (DPPH•, ABTS•+, CUPRAC ve metal şelatlama aktivitesi) belirlenmesi amaçlanmıştır. Süperkritik ekstrelerin (OE1-OE2) TPC ve TFC değerleri sırasıyla 2418,80±102,1 – 3951,46±123,7 µg GAEs/mg ekstre ve 384,61±16,8 – 491,70±27,3 µg QEs/mg ekstre olarak hesaplandı. DPPH• radikal süpürücü aktivite hariç çalışılan *in-vitro* antioksidan testlerin hepsinde hem OE1 hem OE2 süperkritik ekstreleri önemli düzeyde aktivite gösterdi. ABTS•+ radikal süpürme aktivitesi OE2 superkritik ekstresinde (IC_{50}: 30,13±0,82 µg/ml) OE1 superkritik ekstresinden (IC_{50}: 47,21±0,36µg/mL) daha yüksek bulundu. OE2 superkritik ekstresinde CUPRAC aktivitesi ($A_{0.50}$: 206,52±0,24 µg/mL) OE1 superkritik ekstresinden ($A_{0.50}$: 256,71±0,13µg/mL) daha yüksek bulundu. Metal kelatlama aktivitesi ise OE2 superkritik ekstresinde (IC_{50}: 26,26±0,72 µg/mL) OE1 superkritik ekstresinden (IC_{50}: 30,17±0,53 µg/mL) daha yüksek gözlendi. DPPH• radikal süpürücü aktivite, *O. europaea* L.'nin iki ekstraktında da önemli bir aktivite göstermedi. Dolayısıyla süperkritik CO_2 ekstraksiyon yönteminde 300 bar basınç altında ekstrakte edilen (OE2) *O. europaea* subs. *oleast*er yapraklarının daha yüksek TPC/TFC ve in-vitro antioksidan aktivitelere sahip olduğu gözlendi. Sonuç olarak, *O. europaea* subs. *oleast*er yaprak ekstraktlarının fenolik bileşikler açısından çok güçlü bir zenginliğe sahip olduğu ve flavonoidlerin çok ilginç antioksidan potansiyele sahip oldukları gözlendi. Ayrıca gıda koruyucu olarak farklı alanlarda kullanılabilecek önemli fenolik bileşik olabileceğini, sentetik antioksidanlar için *O. europaea* subs. *oleast*er yapraklarının önemli bir alternatif oluşturabileceğini düşünüyoruz.

Olea europaea L. subs. *oleaster* Yapraklarının Süperkritik CO_2 Ekstraksiyonuyla Fenolik İçerik ve *İn-vitro* Antioksidan Aktivitesi

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ABSTRACT

In the study, we aimed to define total phenolic (TPC) and flavonoid (TFC) contents and antioxidant invitro assays (DPPH•, ABTS•+, CUPRAC and metal chelating activity) of supercritical CO_2 extracts obtained by supercritical CO_2 extraction method (SCFE) under pressure of 150 bar and 300 bar of O. europaea subs. L. oleaster leaf, which grows naturally in the Tarsus district of Mersin province, in the Mediterranean Region, has an important economic potential. TPC and TFC of the supercritical samples (OE1-OE2) were predicted as 2418,80±102,1 – 3951,46±123,7 µg GAEs/mg extract and 384,61±16,8–491,70±27,3 µg QEs, respectively. Except for DPPH• radical scavenging activity, both OE1 and OE2 supercritical extracts showed significant activity in all in-vitro antioxidant tests studied. ABTS•+ radical scavenging activity in the OE2 supercritical extract (IC50: 30,13±0,82 µg/ml) was determined higher than OE1 supercritical extract (IC50: 47,21±0,36µg/ml). CUPRAC activity in OE2 supercritical extract (A0.50: 206,52±0,24 µg/mL) was found higher than OE1 supercritical extract (A0.50: 256,71±0,13µg/mL). Metal chelating activity value of OE2 supercritical extract (IC50: 26,26±0,72 µg/mL) was found higher than OE1 supercritical extract (IC50: 30,17±0,53 µg/mL). DPPH• assay showed not any remarkable activity in extract of O. europaea subs. Oleaster. Therefore, it was observed that O. europaea leaves extracted under 300 bar pressure in the supercritical CO_2 extraction method (OE2) had higher TPC/TFC and in-vitro antioxidant activities. As a result, it was observed that O. europaea subs. oleaster leaf extracts had a very strong richness in terms of phenolic compounds and flavonoids had very interesting antioxidant potential. So, we proposed that O. europaea subs. oleaster leaves can be a valuable source of phenolic compounds as a food preservative in different fields, and that O. europaea subs. oleaster leaves can be an important alternative for synthetic antioxidants.

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INTRODUCTION

The *Olea europaea* L. (Olive), belonging to the Oleaceae (Olive family), is divided into two subspecies: *O. europaea* subs. *sativa* (cultivated olive) and *O. europaea* subs. *oleaster* (wild olive). *O. europaea*, widely distributed across tropical and temperate regions worldwide, naturally prospers in maquis habitats characterized by the prevalence of the Mediterranean climate and is cultivated in countries within this region (Baytop, 1999; Ghanbari, Anwar, Alkharfy, Gilani, & Saari, 2012). Approximately 98% of the olive trees, which cover more than 8 million hectares worldwide, are located in the Mediterranean basin (Talhaoui et al., 2014). In Turkey, located within the Mediterranean basin, olives are an important product due to their economic value and production volume (Bedestenci & Vuruş, 2000).

Oleuropein is notably the prominent phenolic compound found in various olive cultivars and can be easily extracted from the phenolic fraction of olive parts (Talhaoui et al., 2014). Due to the presence of oleuropein extracted from the fruits and leaves of the olive plant, it is deemed highly significant pharmacologically (Omar, 2010). Due to the positive health effects of oleuropein, its main bioactive component, the olive leaf has been recognized as an herbal medicine by the European Medicines Agency (EMA)'s Committee on Herbal Medicinal Products (HMPC) (Anonymous, 2011).

In Mediterranean countries, the olive leaf is commonly utilized in traditional treatments (Anonymous, 2011). In these countries, there is a growing belief that the olive leaf, in addition to olive oil, might contribute to the reduced incidence of certain diseases (Armutcu, Akyol, Hasgul, & Yigitoglu, 2011). In our country, the leaves are used internally as a 5% infusion to stimulate appetite, act as a diuretic, relieve constipation and reduce fever. It is also used for diabetes. Externally, it is employed for cleansing inflamed wounds and in their dressing (Baytop, 1999). Although plants are evaluated for the treatment of different illnesses (Colcimen, Arihan, Gumusok & Kilic, 2020), it is reported that their indiscriminate consumption can lead to serious health problems, especially in children (Karakuş, 2018). This situation necessitates the active involvement of preschool teachers both in the context of safeguarding the child's health and in instilling positive health behaviors to foster a healthy lifestyle in the child (Bodur, Durduran, & Küçükkendirci, 2012; Özgür & Ekuklu, 2018; Sayar, 2020).

Fundamentally, since each botanical material possesses its unique characteristics, developing extraction processes under specific conditions for each material is of utmost importance to ensure the procurement of safe products, free from hazardous substances or adverse effects that could jeopardize consumer health and well-being, and also to achieve high efficiency and to keep the quantity of organic solvents to a minimum (P. Putnik, Kovačević, Radojčin, & Dragović-Uzelaca, 2016). The success of extraction is dependent on numerous factors, including the extraction technology employed, the thermal stability of the targeted bioactive compounds for recovery, the duration of the extraction process, and the type of solvent used (Predrag Putnik et al., 2018; Roselló-Soto et al., 2015).

The most frequently declared extraction technique for polyphenols is solid-liquid extraction carried out through mechanical agitation with various organic solvents (Ameer, Shahbaz, & Kwon, 2017; Tsimidou & Papoti, 2010). When reviewing the literature, it becomes evident that many researchers commonly employ ethanol, ethyl acetate, methanol, acetone, and their aqueous solutions to extract polyphenols (Contini, Baccelloni, Massantini, & Anelli, 2008; Rosa et al., 2019). However, extractions conducted with these solvents may result in adverse effects such as low yields, thermal degradation, and substantial consumption of organic solvents (Predrag Putnik et al., 2018).

The increasing interest in both nutrition and herbal treatment methods in recent times has led to a growing fascination, particularly in olive leaves, particularly due to their richness in phenolic compounds. Olive oil is a highly significant product due to its varying amounts of triacylglycerols and small quantities of free fatty acids, pigments, sterols, glycerol, tocopherols, aroma compounds,

resinous components, and phenols (Omar, 2010). Olive leaves possess a significant amount of useful bioactive components within their chemical structure. Olive leaves contain a wide range of phenolic derivatives, comprised of flavonoids, secoiridoids, and simple phenols (Anonymous, 2011). Due to the phenolic compounds present in olive leaves, they possess antimicrobial, anti-inflammatory, antiatherogenic, antihypertensive, hypocholesterolemic, hypoglycemic, antiviral, antioxidant, and antitumoral properties (Armutcu et al., 2011; Özcan & Matthäus, 2017). Furthermore, researchers have expressed that olive leaf extracts are exceptional antioxidants and could offer as an alternative to synthetic antioxidants (Armutcu et al., 2011).

As an alternative, in this context, both pressurized liquid extraction (PLE) and supercritical fluid Extraction (SFE) are the most commonly employed techniques which are based on the utilization of compressed liquids to obtain bioactive compounds (Herrero, Castro-Puyana, Mendiola, & Ibañez, 2013). Due to their low (or zero) consumption, processes of this nature are widely recognized as green technologies. The most crucial stage in accurately determining the phenolic composition of olive leaves is the extraction phase of phenolic compounds. The type and ratio of solvent, which vary based on the extraction methods for phenolic compounds, along with factors such as pH, temperature, and sample particle size, directly impact the extraction efficiency (Abaza et al., 2011). In studies focusing on olive leaves, it has been observed that various drying methods are applied to the leaves prior to extraction (Margarita H. Ahmad-Qasem et al., 2016, Bahloul, Boudhrioua et al.,2009). During the extraction phase, various methods are employed such as pressurized liquid, ultrasound-assisted (Margarita Hussam Ahmad-Qasem et al., 2013), microwave-assisted (Rafiee, Jafari, Alami, & Khomeiri, 2011; Taamalli et al., 2012) and supercritical fluid extraction (Le Floch, Tena, Ríos, & Valcárcel, 1998).

The present study investigated the olive leaves of the *O. europaea* subs. *oleaster* (wild olive) variety, which naturally grows in the Tarsus district of Mersin province within the Mediterranean Region and holds significant economic potential. The aim was to predict the TPC and TFC compounds with the invitro antioxidant activities in the extracts obtained by the supercritical CO_2 extraction method at various pressures.

MATERIALS and METHODS

Plant Material and Collection

The plant samples of *Olea europaea* L. subs. *oleaster* species which belong to the genus *Olea* L. were collected from the Mediterranean region of Türkiye (Tarsus/Mersin). The samples of the mentioned plant were collected fresh from the province of Çokak village, where it is naturally distributed, and dried in a moisture-free environment without exposure to sunlight. Some of the collected plant samples were prepared to be herbarium samples, and their observable features in the area were also recorded. Herbarium samples belonging to the plant are kept at the Necmettin Erbakan University Ahmet Keleşoğlu Faculty of Education Biology Education Herbarium. Herbarium records of the plant were enrolled as C4: Mersin: Tarsus, Çokak Village, Han location, slopes, 390m, 19.08.2022, S. Doğu 4023. Identification of the *Olea* species which used in our study was made by Assoc. Prof. Dr. Süleyman DOĞU.

Chemicals

The chemicals used in the study were at analytical level. Quercetin, sodium carbonate, Folin-Ciocalteu reagent, gallic acid, acetic acid, hydroxytyrosol, acetonitrile, DPPH, and Trolox, were purchased from Sigma/Aldrich (Germany).

Experimental of Supercritical (SCO_2) Extraction (SCFE)

Collected and dried samples of *Olea europaea* L. subs. *oleaster* species was ground up to 0.55 mm size with a model chopper device of EMR-0-01, 28000 r/m, Emir, Istanbul, Türkiye. The ground

samples were processed with 'P-25 35L Super Critical CO_2 Extractor System' device, (Nantong Borisbang Industrial Technology Co., Ltd, BIT HUAAN) at 150 bar and 300 bar at 60 ^{0}C for 2.5 hours. The samples were stored in the refrigerator until the analysis day.

TPC and TFC measurement

According to the Folin Ciocalteu method, *O. europaea* subs. *oleaster* supercritical extracts quantified for TPC (Slinkard & Singleton, 1977) (Slinkard & Singleton, 1977). At 750 nm wavelength, absorbance was measured using a UV/vis spectrophotometer (Biochrom, England). Data were assessed using the formula measured from the std gallic acid graph:

absorb=0.0123 [gallic acid (µg),]-0.0155, (r2, 0.9931)

TFC of the *O. europaea* subs. *oleaster* supercritical extracts was measured via aluminum nitrate method (Park, Koo, Masahuru, & Contado, 1997) (Park et al., 1997). Obtained value was predicted via UV-vis spectrophotometer (Biochrom, England) at 415 nm. Results were calculated using the std quercetin as:

Absorb=0.0156 [quercetin (µg),] - 0.0112 (r2, 0.9985)

Antioxidant Assays

Antioxidant activities of the *O. europaea* subs. *oleaster* supercritical extracts were tested using DPPH•, ABTS•+, CUPRAC and metal chelating activity assays (Keskinkaya et al., 2022). BHT, BHA and EDTA were applied as standards. The IC50 value (50% inhibition) was measured via graph/plotted between the antioxidant activity and the concentration of the samples.

The A 0.50 value (0.50 absorb) was measured via plotted between the concentration and absorbance of the samples. Values were presented as IC50 values and inhibition (%) at 400 µg/mL concentration for DPPH•; A0.50 values and absorbance (400 µg/mL) concentration CUPRAC.

DPPH

O. europaea subs. *oleaster* supercritical samples were determined via DPPH• (Blois, 1958) (Blois, 1958). 0.4 mM of DPPH (160 µL) was put to 40 µL of sample at different concentrations. (as a control, 40 µL of methanol, 30 min/dark). The value was predicted at 517 nm. The DPPH was measured using the equation as (1):

DPPH (% Inhibition) = (A_control-A_Sample)/A_Control x 100 (1)

AControl: absorb of the control, Asample: absorb of the sample.

ABTS

ABTS uses cation radical to predict the free radical scavenging activities of the *O. europaea* subs. *oleaster* supercritical extracts (Re et al., 1999). ABTS•+ (160 µL) was added to 40 µL of sample at different concentrations (10 min, at room C0) were incubated and data was predicted at 734 nm (as control, 40 µL of methanol). ABTS was measured via equation (2):

ABTS·+ scavenging activity (% Inhibition) = (A_Control-A_Sample) / A_Control x 100 (2)

CUPRAC

The decline properties of the *O. europaea* subs. *oleaster* supercritical samples was predicted via the copper (II) ion reducing antioxidant capacity method (Apak, Güçlü, Özyürek, & Karademir, 2004). 10 mM Cu (II) 50 (µL), 7.5 mM neocuproin (50 µL) and ammonium acetate buffer 1 M (60 µL), pH=7 solutions were put into 40 µL of algae sample of various concentrations and (1 hour incubation) and incubated with 96 wells. Absorbances was determined at 450 nm.

Activity of Metal Chelating

Decker and Welch (1990) method was used to assess the chelating activities of the *O. europaea* subs. oleaster samples extracted supercritically.

The reaction was started by adding 40 µL of 0.2 mM $FeCl_2$ solution, 40 µL of ethanol, and 80 µL of 0.5 mM ferrin to 40 µL of sample solutions of different concentrations. The mix kept at room temperature for 10 min. Finally, absorbance was determined at 593 nm. The metal binding activity was measured via the equation in (3):

Metal chelating activity (% Inhibition) = x 100 (3)

Statistical Analysis

The data collected was analyzed using IBM SPSS Statistics 23.0 for Windows, which is the Statistical Package for Social Sciences. For continuous variables, descriptive analyses were conducted, and the arithmetic mean along with the standard deviation (SD) values of the variables were provided. The analysis results were interpreted with a significance level set at 0.05, and the outcomes were presented with a 95% confidence level.

RESULTS

In our study phenolic content in total flavonoids and radical scavenging *in vitro* assays (DPPH•, CUPRAC, ABTS•+, and metal chelating activity of supercritical CO_2 extracts of *O. europaea* subs. *oleaster* leaves. The leaves grow naturally in the Tarsus district of Mersin province in the Mediterranean region and have an important economic potential. Extraction of the leaves was completed by using the supercritical CO_2 extraction method (SCFE) under pressure of 150 bar and 300 bar.

The results of flavonoids and phenolics of the OE1 and OE2 supercritically extracted materials were given in Table 1. The data is presented as the mean value along with the standard error of the mean (SEM) based on three replicates (n=3). OE2 supercritical extract (3951.46±123.7 µg GAEs/mg extract) was recorded as richer than OE1 supercritical extract (2418.80±102.1 µg GAEs/mg extract) in terms of TPC. OE2 supercritical extract (491.70±27.3 µg QEs/mg extract) was recorded as richer than OE1 supercritical extract (384.61±16.8µg QEs/mg extract) according to the TFC results.

Table 1. *Phenolics in Total (TPC) and Flavonoids (TFC) of O. europaea* subs. *oleaster Supercritical Extracts*[a]

Extracts	**TPC** (µg GAEs/mg extract[b])	**TFC** (µg QEs/mg extract[c])
OE1	2418.80±102.1	384.61±16.8
OE2	3951.46±123.7	491.70±27.3

[a]: The data is given as the mean value along with the standard error of the mean.
OE1: *O. europaea* subs. *oleaster* SCFE extract 150 bar pressure,
OE2: *O. europaea* subs. *oleaster* SCFE extract 300 bar pressure. M
[b] GAEs, Equivalent to gallic acid, y=0,0123x-0,0155 r^2=0,9931
[c]QEs, quercetin equivalent, y=0,0156x-0,0112 r^2=0,9985

Because antioxidants operate through various mechanisms, it is advisable to use multiple methods for assessing antioxidant activity rather than relying on a single approach. We evaluated the antioxidant properties of OE1 and OE2 extracts using CUPRAC, DPPH•, metal chelating assays and ABTS•+. The findings are presented in Table 2.

ABTS in the OE2 supercritical extract (IC50: 30.13±0.82 µg/ml) was determined higher than OE1 supercritical extract (IC50: 47.21±0.36 µg/ml). CUPRAC activity in OE2 supercritical extract (A0.50: 206.52±0.24 µg/mL) was found higher than OE1 supercritical extract (A0.50:

256.71±0.13µg/mL). Metal chelating activity values of OE2 supercritical extract (IC50: 26.26±0.72 µg/mL) were found higher than OE1 supercritical extract (IC50: 30.17±0.53 µg/mL). The DPPH radical scavenging assay demonstrated no substantial activity in any extract of *O. europaea* subs. *oleaster*.

Table 2. *Antioxidant activity of O. europaea* subs. *oleaster extracts.*

		Antioxidant Activity							
		DPPH•		ABTS•+		CUPRAC		Metal Chelating	
		Inhibition (%)[a]	IC_{50} (µg /mL)[b]	Inhibition (%)[a]	IC_{50} (µg/mL)[b]	Absorbance[c]	$A_{0.50}$ (µg/mL)[d]	Inhibition (%)[a]	IC_{50} (µg /mL)[b]
Extracts	QE1	-	>400	72.11±0.29	47.21±0.36	0.73±0.10	256.71±0.13	94.77±0.51	30.17±0.53
	QE2	-	>400	77.80±0.18	30.13±0.82	0.76±0.17	206.52±0.24	90.48±0.67	26.26±0.72
Standards	BHT	87.11±0.29	24.13±0.24	86.71±0.43	13.07±0.49	2.61±0.28	27.64±0.06		
	BHA	87.90±0.22	23.21±0.37	89.03±0.72	12.88±0.31	2.96±0.09	26.95±0.02		
	EDTA							91.16±0.31	4.53±0.19

OE1: *O. europaea* SCO_2 extract 150 bar pressure, OE2: *O. europaea* SCO_2 extract 300 bar pressure.

[a]: The inhibition percentages at a concentration of 400 µg/mL for the extracts are presented as the average ± standard deviation (SD)

[b]: IC_{50} values are reported as the mean ± standard deviation (SD).

[c]: Mean absorbance values at a concentration of 400 µg/mL for the extracts are presented as the mean ± standard deviation (SD).

[d]: Mean $A_{0.50}$ values are reported as the mean ± standard deviation (SD).

DISCUSSION, CONCLUSION

In our study, we assessed the levels of phenolic content in total (TPC) and flavonoid (TFC) compounds, as well as the antioxidant properties *in vitro* using various assays such as ABTS•+, DPPH•, metal chelating activity, CUPRAC. These evaluations were conducted on extracts obtained from the leaves of *Olea europaea* subspecies and oleaster plants through a supercritical CO_2 extraction method (SCFE) at two different pressure levels, 150 bar and 300 bar. These plants naturally grow in the Tarsus district of Mersin province in the Mediterranean Region and hold significant economic potential.

Total phenolic and flavonoid contents of extracted materials (OE1-OE2) by supercritical method were determined as 80±102.1-3951.46±123.7 µg GAEs/mg extract and 384.61±16.8-491.70±27.3 µg QEs/mg extract respectively. In parallel with the literature (Temiz & Temur, 2017; Zhishen, Mengcheng, & Jianming, 1999), it was determined that flavonoids and phenolics content of *O. europaea* subs. *oleaster* leaves were quite high in total.

Extraction methods and solvent exchange elevate the amount of TPC/TFC and improve its antioxidant potential. In a study (Silva, Gomes, Leitão, Coelho, & Boas, 2006), lipids and pigments from different olive leaves were extracted, firstly 3 times with hexane. Then, the polyphenolic compounds were extracted and defined with terms of MeOH/water (4:1 v/v). At the study, polyphenolics are determined at a different olive culture by using the Folin-Denis method and measured between 1.170-4.010 mg 100g-1 tannic acid equivalent. Another research study (Taamalli et al., 2012) evaluated the phenolic compounds in olive leaf according to the results of various techniques of extraction with HPLC/ESI/TOF/MS. Notably, the microwave-assisted extraction

method yielded the highest total phenolic content (TPC) compared to other methods, surpassing the conventional solvent extraction method that used MeOH/water (4:1, v/v) as the solvent. In the study it was predicted that the amount of oleuropein from these two methods was determined to be as highest method (Taamalli et al., 2012).

A different research study (Taamalli et al., 2012) examined the phenolic compounds found in olive leaves using various extraction methods, employing HPLC/ESI/TOF/MS. Notably, the microwave-assisted extraction method yielded the highest total phenolic content (TPC) compared to other methods, surpassing the conventional solvent extraction method that used MeOH/water (4:1, v/v) as the solvent.

Except for DPPH• radical scavenging activity, both OE1 and OE2 supercritical extracts showed significant antioxidant activity in all in-vitro antioxidant tests studied. The findings we obtained are similar to the results reported by many researchers (Sánchez-Gutiérrez et al., 2021, Khlennikov et al., 2007).

In the research carried out by Casado-Diaz et al. (2022) in 2022 to determine the antioxidant activities of *Olea europaea* leaf extract (OELE), it's declared that phenols, flavonoids, and oleuropeosides have an important role against oxidative stress. When combined with the extract OELE phenolic compounds it has a close effect on antioxidant capacity compared with their individual effects. Meanwhile, the ROS scavenging properties of phenols are related to the properties, amounts and hydroxyl position of their functional groups (Lins, Marina Piccoli Pugine, Scatolini, & de Melo, 2018). In the research of Casado-Diaz et al. (2022), the antioxidant properties of OELE which were determined by ABTS, DPPH, and FRAP assays was not reduced by inclusion in the EHO-85 gel formulation, as the results were similar to the amount of extract contained (0.1%). Also, there was a pronounced impact observed in the DPPH assay in contrast to the ABTS and FRAP assays. This result may be related to the nature of OELE, which reacts effortlessly with the DPPH radical (Gordon, Paiva-Martins, & Almeida, 2001). In our study, while the efficacy of oleaster leaf extracts was observed at different levels in ABTS, CUPRAC and metal chelating activity tests (OE1-IC50: 47.21±0.36µg/mL, OE2- IC50: 30.13±0.82 µg/mL; OE1- A0.50: 256.71±0.13 µg/mL, OE1-A0.50: 206.52±0.24 µg/mL; OE1-IC50: 30.17±0.53 µg/mL, OE2- IC50: 26.26±0.72 µg/Ml, respectively). The activity of scavenging radicals of the extracted materials was not observed in the DPPH test.This difference may be due to the different phenolic compounds obtained through the SCFE extraction used and the principles of chemistry on which the antioxidant activity experiments are based on methods. In future studies, there will be an examination of the phenolic profile in *O. europaea* subs. *oleaster* leaf extracts. This analysis will focus on both the quantitative and qualitative assessment of the components responsible for antioxidant properties and their effectiveness in the conducted tests. Gikas et al. reported that the most important source of this compound in nature is the olive leaf, which is a by-product of olive cultivation, although oleuropein, which is found in the whole olive tree, is also found in olives, therefore, in its pulp, oil and wastes (alperujo) that occur during olive oil production (60-90 mg/g dry weight) (Gikas, Bazoti, & Tsarbopoulos, 2007). Studies have shown that the content of oleuropein in olive oil varies between 0.005% and 2%, in alperujo it is 0.87% and in olive leaf it is between 1-14% (Beauchamp et al., 2005).

Our study focused on evaluating the in-vitro antioxidant capabilities of supercritical extracts derived from *O. europaea* subs. *oleaster* leaves through the measurement of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The results of our investigation indicate that these extracts are exceptionally rich in flavonoids and phenolic compounds, in particular, demonstrate significant antioxidant potential, consistent with findings from earlier studies (Baharfar et al., 2015, Tungmunnithum et al., 2018, Huang et al., 2011).

This suggests that these extracts could serve as a valuable source of phenolic compounds

suitable for use in various applications, such as preservation of foods and developing products in pharmaceutical sectors and various industrial applications.

As a result, there are multifaceted studies on the extraction of phenolics from plants, the use of the biological potential of wastes is mainly restricted to antioxidant activity and antimicrobial activity (Dai et al., 2010). The study aimed to evaluate various unexplored biological activities of bioactive substances which extracted from plants using SCFE. Nowadays, application of SCFE for the extraction of bioactive substances from plant materials and fruit wastes has not been exactly realized and has been restricted to applications in the food industry. However, the significant advantages of SCFE offers a low operating cost and a greener eco-friendly extraction approach in the related industry.

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