Scientific Journal of Agricultural Sciences

Print (ISSN 2535-1796) / Online (ISSN 2535-180X)

Determination and Identification of The Biologically Active Compounds in Olive Leaves

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Citation: Shaymaa N. Zayed, Ahmed A. Ashour, Mohamed Abd Alim, Mohamed E. Hassan and Enas M. Mekawi (2024). Determination and Identification of The Biologically Active Compounds in Olive Leaves. Scientific Journal of Agricultural Sciences, 6 (2): 123-137. https://doi.org/10.21608/sjas.2024.284093.1412.

Publisher :
Beni-Suef University, Faculty of Agriculture

Received: 20 / 4 / 2024
Accepted: 18 / 6 / 2024

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ABSTRACT

Olive leaf extract (OLE) is one of the natural extracts utilized to obtain bioactive components. It contains the potential sources of several phenolic compounds. The total flavonoid concentration, total phenolic content, antioxidant activity, and drying procedures of OLE, air-dried (AD), freeze-dried (FD), and fresh extract (FE) were evaluated via HPLC. LC–MS/MS characterization and identification were subsequently conducted to obtain additional structural information on the separated compounds. The extract obtained using ethanol (80%) had a high total phenolic content (114.55 FD, 91.95 AD, and 63.41 mg FE/g dw) and TFC (251.25 FD, 254.43 AD, and 111.67 mg FE/g dw). Additionally, the DPPH technique was used to assess the antioxidant activity of OLE (82.43 FD, 89.53 AD, and 64.13 mg FE/g dw). HPLC was used to characterize and identify 21 bioactive compounds. Phenolic compounds such as Oleuropein, Apigenin-7-O-glucoside, Luteolin-7-O-glucoside, Luteolin, Quercetin, Homo vanillic acid, Apigenin, Hydroxytyrosol, Caffeic acid, Tyrosol, and Vanillin were identified via the LC–MS/MS profile of OLE. The results of this study indicate that the bioactive components obtained from olive leaf extract are valuable sources of antioxidants, which may lead to applications in the pharmaceutical and food industries.

KEYWORDS: Olive leaves; Antioxidants; Total phenolic content; Radical scavenging activity; LC. MS/MS analysis

1. INTRODUCTION

Plant extracts and byproducts with high concentrations of bioactive chemicals have attracted increased interest from the pharmaceutical, cosmetic, and agronomic sectors (Joana et al., 2013). This is due to the growing demand for natural preservatives and the desire to create new functional foods with significant health benefits (Al-Okbi et al., 2018; Ribeiro et al., 2015).

One of the most widespread and traditio nal types of fruit trees is the olive (OL) type. Olive leaves (OLs) have been utilized in a

number of studies. Among them are thousands of varieties. According to Gargouri et al., (2013), these species have evolved to be well suited to a variety of environmental conditions.

The Mediterranean region is home to approximately 8 million olive trees, which is estimated to be 98% of all olive trees that grow worldwide (Peralbo-Molina et al., 2013). However, olive leaves make up 10% of the aggregate weight of harvested olives. Recycling these waste resources, which are available, is crucial, particularly if they have high economic value. As a result, minimizing and eliminating waste will improve both the economy and the environment (El and Karakaya, 2009). Conversely, phenolic compounds, which are abundant in this biomass, have a strong capacity to scavenge radicals (Nassar et al., 2016; Altıok et al., 2008; Japón-Luján et al., 2008; Mavrakis, 2009).

Its antiviral, anticancer, and antioxidant activities are among the most significant advantages of naturally occurring compounds with biologically active characteristics (Benavente-García et al., 2000; Liu et al., 2003). Considering the negative effects of synthesized antioxidants, natural antioxidants have gained significant interest (Olmedo et al., 2015). An alternative strategy is to use plant-based extracts, essential oils extracted from herbs and spices, and natural antioxidants such as tocopherol. The aforementioned substances and integrates have been used to create antioxidant packaging materials (Marcos et al., 2014; Li et al., 2014; Licciardello et al., 2015; Akrami et al., 2015).

In addition to their traditional use as animal feed, olive leaves can be found in several higher-value industries, such as food, cosmetic, and pharmaceutical industries. Olive leaves contain a wide range of nutrients, including sugars, iridoids, flavones and flavonoids. According to Bayçin et al., (2007) and Dekanski et al., (2009), leaves contain phenols, which can be found in a range of forms and concentrations, making them a potential source of bioactive chemicals. Hence, Tsimidou et al., (2010) assembled data pertaining to major molecular properties, such as simple phenols and acids; lignans; flavonoids, such as rutin, oleeuropein, secoiridoids, flavanols, and catechin; flavones, such as luteolin-7-glucoside, luteolin, diosmetin-7-glucoside, apigenin-7-glucoside, and diosmetin; and substituted phenols, such as hydroxytyrosol, vanillin, vanillic acid, Tyrosol, and Caffeic acid. El et al., (2009) and Kontogianni et al., (2012) reported that the primary constituents of olive leaves are Secretoiridoids linked to Oleuropein and its derivatives.

In laboratory (in vitro) and in vivo studies, oleuropein, the primary phenolic secoiridoid found in *OleaeuroPaea L. (Oleaceae*), has been shown to be a strong antioxidant (Mylonaki et al., 2008; Koca et al., 2011). According to research conducted on rats given a high-fat, high-carb diet, oleuropein, or olive leaves, oleuropein protects the heart from ischemia and reperfusion and improves cardiac, hepatic, and metabolic changes (Manna et al., 2004; Poudyal et al., 2010). Plant tissues contain polyphenols, which are essential for several processes including pigmentation, growth, reproduction, and disease resistance (Lattanzio et al., 2006).

The bitter flavours of table olives and extra virgin olive oil come from oleuropein, the major polyphenolic compound found in olive leaves. Numerous investigations have shown that oleuropein has a variety of antiviral, antibacterial, antioxidant, and anti-inflammatory effects (Alzweiri and Al-Hiari, 2013; Hayes et al., 2011). Research has indicated that oleuropein is a significant biophenol that has anticancer activity in addition to its anti-inflammatory, antihypertensive, antiviral, antiatherogenic, and cardioprotective effects. Due to their strong antioxidant qualities, these compounds, which are members of one of the main families of secondary metabolites, have recently gained much attention. Olive leaves are a cheap and readily accessible natural resource; indigenous people in the Mediterranean region have employed their extracts in traditional medicine (Visioli and Galli, 1998; Singh et al., 2008; Benavente-García et al., 2000). This unique characteristic of olive leaves is caused by their high content of polyphenols, including luteolin-7-glucoside, apigenin-7-glucoside, verbacoside, rutin, and oleuropein (Savournin et al., 2001).

According to Qabaha et al., (2018), the primary constituent of olive leaf extracts, oleuropein, is responsible for the biological

effects of the extracts. Olive leaves include a variety of other compounds, such as luteolin, apigetrin, luteolin-7-O-glucoside, and apigenin, all of which have been linked to health benefits. Consequently, recent research has shown that OL has high value due to its excellent antioxidant qualities, high phenolic component content, and ability to assess antioxidant activity (Martins et al., 2007; Kiritsakis et al., 2010; Xynos et al., 2012; Malheiro et al., 2013). Most investigations have determined that oleuropein is the main phenolic component in OL. Oleuropein has a significant positive impact on the technical and nutritional qualities of plants, including its anti-inflammatory and antioxidant effects, which may be linked to its ability to prevent certain diseases such as atherosclerosis. It also possesses antibacterial and antihypertensive properties (Huang and Sumpio, 2008; Nekooeian et al., 2014; Khalatbary and Zarrinjoei, 2012; Susalit et al., 2011). Lutein-7-O-glucoside is another significant phenolic component found in OL and is a powerful medication that prevents colon cancer from developing. Thus, considering the increasing need for natural additives to replace chemical additives, olive leaf extracts (OLEs) might be viewed as a significant, easily accessible, and moderately priced raw resource that can be considered a natural antioxidant (Romero-García et al., 2014; Lama-Muñoz et al., 2019). Therefore, the primary objective of the current study was to estimate the antioxidant activity of phenolic compounds extracted from olive leaves. The olive leaf extract was characterized by HPLC and LC–MS/MS analysis.

2. MATERIALS AND METHODS

2.1. Materials

Fresh olive leaf (OL) samples were collected on two sampling dates in different time mostly average between mid-march and mid-june, 2021 and 2022, at the faculty of agriculture at Benha University (Egypt). Folin-Ciocalteu phenol reagent, gallic acid, and quercetin were obtained from Sigma–Aldrich Chemical Company and were of high quality and purity. 2,2-Diphenyl-1-picryl hydrazyl (DPPH; M.W. 394.32; assay 95%; melting point 125–145 °C) was purchased from Sisco Research Lab Srichem Co. (India).

2.2. Methods

2.2.1. OL extract preparation (OLE):

Olive leaves were carefully plucked, removed from branches, and cleaned carefully with tap water and then distilled water. OLs were divided into three groups: one group was dried by a lyophilization process (freeze-dried sample), the other group was dried at room temperature (air-dried sample), and the third group remained fresh. All leaves were ground into a fine powder by using liquid nitrogen to keep the bioactive compounds safe from any environmental conditions; any impurities were removed, and the leaves were stored in light-protected Falcon tubes in a refrigerator until analysis for experimental use. The 80% (The ratio of dried plant to solvent is 1:10 w/v) ethanol extract was made in a dark area and thoroughly mixed each day. After that, the samples were passed through Whatman filter paper to filter the combined ethanol extract.

2.2.2. Chemical composition analyses of Olive leaves (OL).

Moisture, Carbohydrates, Ash, Proteins, and total Lipids were determined according to (AOAC, 2019) .

2.2.3. Total phenolic content of the extract.

The total phenolic content of the OLE ethanolic extract was determined using the Folin-Ciocalteu assay in compliance with the methods of Singleton and Rossi (1965) and John et al. (2014). It was given as milligrams of gallic acid equivalents (GAE) per sample's dry weight.

2.2.4. Determination of the total flavonoid content

According to Zhishen et al. (1999), the total flavonoid content in the OLE ethanolic extract was determined using the aluminum chloride colorimetric technique. The total flavonoid concentration was calculated as milligrams of quercetin equivalents (QE) per dry weight of the sample. Quercetin was used as the standard in the creation of the calibration curve.

2.2.5. Determination of antioxidant activity by DPPH

The antioxidant activities of the olive leaf extracts (OLEs) were assessed by scavenging stable DPPH radicals using the Hatano et al., 1988 strategy, which has been used to evaluate the antioxidant activity of various extracts, such as the free radical scavenging activities of antioxidants (RSAs). To calculate the percentage inhibition of DPPH radical scavenging ability, the following formula was utilized:

(RSA%) = [A0-A1)/A0] X 100.

where A0 represents the control absorbance and A1 represents the sample absorbance.

2.2.6. Phenolic compound characterization by HPLC.

Schneider (2016) provided guidelines for HPLC characterization, which were carried out with some performance adjustments utilizing the Agilent 1260 infinite HPLC Series (Agilent, USA), which has a quaternary pump. An Akinetex® 5 µm EVO C18 100 mm*4.6 mm (Phenomenex, USA) column was utilized, and the column was run at 30 °C. A ternary linear elution gradient comprising (A) HPLC-grade water (0.2% H_3PO_4 v/v), (B) methanol, and (C) acetonitrile was used for separation. A 20 µl injection volume was used. The VWD detector was tuned to 384 nm for detection.

2.2.7. Phenolic compound characterization by LC. MS/MS.

A complete scan in the m/z range of 100–1000 was carried out at a rate of one spectrum per second, and MS/MS was then conducted. Fragmentation at varying impact energy levels of the most energetic precursor ions (single charge). It was discovered that the olive leaf extract contained phenolic chemicals using a straightforward liquid chromatography–tandem mass spectrometry (4000 Q-TRAP LC–MS/MS) approach (Benincasa et al., 2018; Ammar et al., 2017). Through direct infusion using a Harvard 11plus pump at a flow rate of 0.1 mL/min and an isocratic mobile phase composed of water, methanol, and formic acid (50:50:0.1 v/v/v), LC/MS optimization was carried out.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of olive leaves (OL):

The chemical composition of the olive leaves is shown in Figure 1. Olive leaves contain moisture (48.54%), protein (9.31%), ash (6.38%), and carbohydrates (35.41%). The findings of Boudhrioua et al., (2009), on the other hand, are consistent with our results. They discovered in the fresh olive leaves of Chemlali, Chemchali, Zarrazi and Chetoui varieties were obtained from the farm of the Olive Institute of Sfax (Tunisia) between March and April 2007 that the moisture content, measured in grams per 100 grams of fresh leaves, ranged between 46.24% and 49.75%, protein content between 5.04% and 7.61%, the fat content between 1.05% and 1.30%, the ash content between 2.86% and 4.45%, and the carbohydrates content between 37.14% and 42.60%. Kashaninejad et al., (2020) determined the total carbohydrate content to be 30%, the ash content to be 4.69%, the lipid content to be 2.7%, and the protein content to be 10.3%. Gullón et al., (2018) studied the olive mill leaf (OML) biomass, sample that was collected in January 2016 from the olive cleaning line at the oil mill and also located in the province of Jaén (Spain) and reported that the total carbohydrate content was 22%, the ash content was 6.9%, and the protein content was 6.9%.

3.2. The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (TAA) were determined via DPPH in OLE.

The extraction of bioactive components from OLE was assessed using three different drying methods: freeze-dried extract (FD), air-dried extract (AD), and fresh extract (FE) produced with ethanol at a concentration of 80%. Figure 2 represent a comparison of the total phenolic content (TPC), total flavonoid content (TFC), and DPPH values.

3.2.1. Total phenolic content

According to these data, the total phenolic content was 114.55 mg/g dw for the FD extract, 91.95 mg/g dw for the AD extract, and 63.41 mg/g dw for the FE extract as gallic acid equivalents. It was reported that the total phenolic content ranged from 12.36 to 27.54 mg/g dw for olive mill leaves (OML) (Gullón et al., 2018). According to Alaaeldin et al., (2021), gallic acid accounts for 53.75 mg/g dw of the total phenolic content of the AD extract. In

addition, research by Mbarka et al., (2018) reported that the phenolic chemical

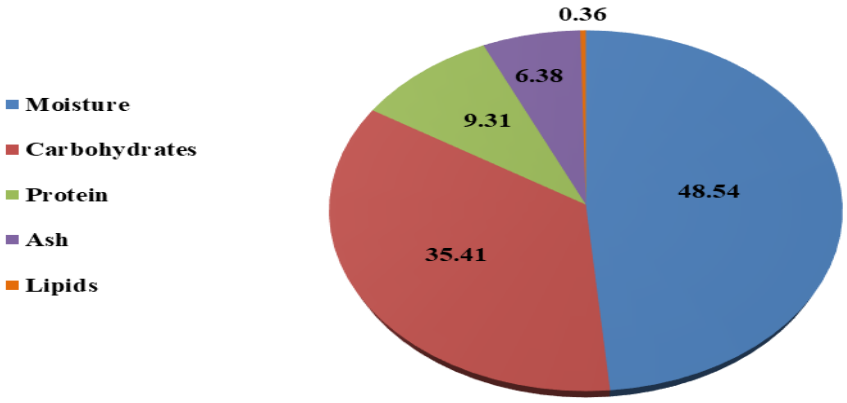


Figure 1. Schematic representation of the chemical composition of olive leaves.

concentration of OLE ranged from 1352 mg/kg to 20662 mg/kg. The findings of these studies are consistent with earlier research (Quirantes-Piné et al., 2013 and Talhaoui et al., 2015), which suggested that olive leaves can be a rich source of phenolic compounds.

3.2.2. The total falvonoid content

In the present study, the total flavonoid content (TFC) of the FD extract was 251.25 mg/g, that of the AD extract was 254.43 mg/g, and that of the FE extract was 111.67 mg/g. According to Gullón et al., (2018), the TFC of OML ranged from 17.89 to 52.39 mg/g dw. Furthermore, Alaaeldin et al., (2021) reported that dried olive leaves contain 32 mg/g flavonoids overall.

3.2.3. Antioxidant activity

DPPH was used to assess the total antioxidant activity of the olive leaf extract. The DPPH free radical scavenging data demonstrated the antioxidant activity of the olive leaf extract. The FD extract, AD extract, and FE OLE had the highest DPPH radical scavenging activity (82.43%, 89.53%, and 64.13%, respectively). According to Alaaeldin et al., (2021), the OL extract exhibited a strong scavenging activity of 42.9 µg/ml against DPPH radicals. Furthermore, Cör et al., (2022) reported that maximum inhibition rates of 98% and 86% in leaves dried at room temperature were detected in supercritical extracts of freeze-dried Istrska belica leaves.

3.2.4. Comparison between TPC, TFC, and TAA.

the yield of ethanolic freeze dried OLE was 96 g extracted from 370g of fine powder; Alaaeldin et al., (2021) and Cör et al., (2022) also noted that high concentrations of some active components are not always linked to high DPPH activity. The disintegration of compounds may be the reason for the difference between the total flavonoid content and the antioxidant activity (DPPH) of the FD and AD extracts in the present study.

Using four distinct drying procedures (vacuum drying, oven drying, freeze drying, and air drying), Filgueira et al., (2022) evaluated the total antioxidant activity (TAA), total phenolic content (TPC), hydroxytyrosol (HC), and oleuropein (OC) in olive leaves (*Olea europaea L.*). It was also determined how these various drying methods affected the TPC, TAA, HC, and OC.

The olive leaves that had been freeze- and air-dried had the greatest TPC and TAA stability. However, air-drying produced more organic carbon (OC) (14–40 mg/g dw) than did freeze-drying (3–20 mg/g dw). After being air-dried, the ecological "Arbequina" leaves had the highest TPC and TAC values. Cör et al., (2022) utilized three different drying methods for two cultivars of olive leaves (Istrska belica and Leccino): air drying in the dark at room temperature for 90 minutes, air drying at 105 °C, and freeze-drying (lyophilization). They found that the highest extraction yield was obtained by

air drying at room temperature, followed by lyophilization.

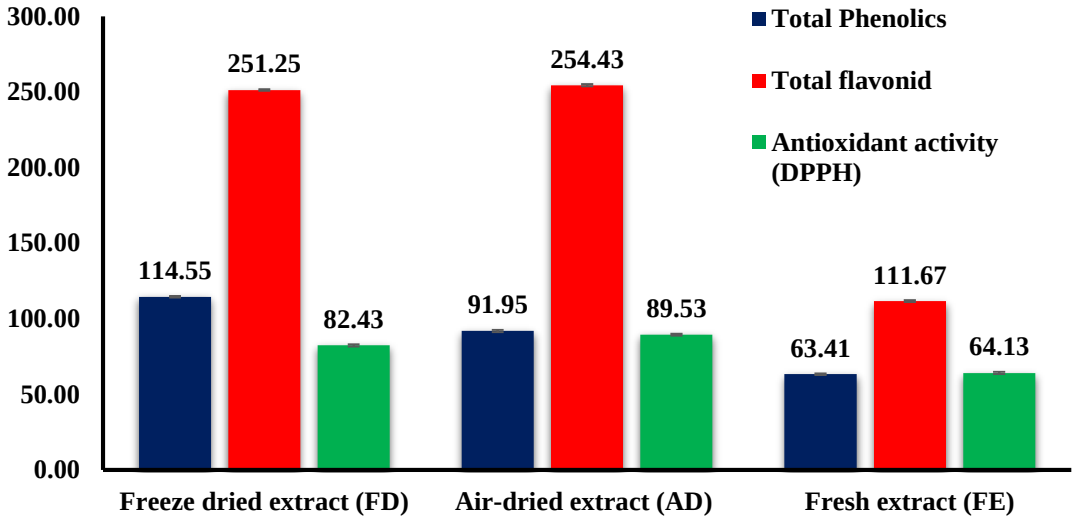


Figure 2. Schematic representation for comparison of the standard deviation and mean values of the extracts from olive leaves that were freeze-dried, air-dried, and fresh, as well as the total phenolic compounds (TPC), total flavonoid compounds (TFC), and total antioxidant activity content (TAA).

3.3. Olive leaf extract characterization

3.3.1. HPLC characterization

According to Talhaoui et al., (2015) and Salido et al., (2015), extracts from olive leaves are rich in phenolic compounds, which include simple phenols, flavonoids, and secoiridoids. This study used HPLC analysis to determine the phenolic content of olive leaves. Table 1 summarizes the data analysis from the HPLC profile and identifies various components of total phenolic compounds in olive leaves along with their molecular formula, quantity, and retention time (RT). Figure 3 displays the 21 major phenolic compound peaks. OL contains the twenty-one chemicals listed below. OL contains a number of different substances, including 3-hydroxytyrosol, catechol, kampherol, P-hydroxybenzoic acid, vanillic acid, catechin, caffeic acid, benzoic acid, gallic acid, syringic acid, P-coumaric acid, ferulic acid, rutin, elagic acid, resvertol, cinnamic acid, myricetin, quercetin, rosemarinic acid, neringein, and o-coumaric acid.

Using a validated HPLC-UV approach, Alaaeldin et al., (2021) identified and quantified the polyphenol from OLE. The five greatest peaks of rutin, apigenin, gallic acid, and oleopin were compared in terms of UV species and retention times under comparable analytical circumstances. On the other hand, Cör et al., (2022) utilized the HPLC-DAD method for identifying the compounds present in olive leaves. Our primary focus was on the chemical oleeuropein. The mass of the master compound oleuropein (mg/g dw) was determined. The extracts contained satisfactorily described components, including oleuropein (OLE), hydroxytyrosol (TyrOH), luteolin-7-glucoside (Lu-7-O-Glu), hydroxy oleuropein (OH-Ole), ligstroside (Lig), verbascoside (Ver), and oleuroside (Ols).

3.3.2. Identification by LC–MS/MS analysis:

The bioactive compounds in the olive leaves were determined by using 4000 QTRAP LC–MS/MS analysis. LC–MS/MS revealed several major phenolic compounds, as shown in Table 2. Eleven secondary metabolites (11 compounds) were characterized and detected (11 peaks), as shown in Figure 4, confirming that olive leaf extracts contain a significant number of bioactive compounds. The first quadrupole (Q1) yielded the molecular ion (m/z) for each chemical, whereas the third quadrupole (Q3) yielded the main fragments. The product ion fragmentation (quantitative analysis) of the compounds was determined to be at m/z. Every compound has an affinity for its molecular formula, primary MS/MS fragments, and

experimental m/z. Oleuropein, an ion at m/z 539; an ion at m/z 447; an ion at m/z 447; an ion

Table 1. HPLC phenolic profile of olive leaf extract.

Peak number	Compounds	Rt (min)	Amount (mg/L)	Percent (%)
1	**"nd"**	-	-	-
2	**"nd"**	-	-	-
3	**Gallic acid**	3.744	0.36222	0.05
4	**3-Hydroxytyrosol**	4.684	428.0336	60.57
5	**Catechol**	5.463	2.99216	0.42
6	**P- Hydroxy benzoic acid**	7.567	35.85978	5.07
7	**Catechin**	9.08	0.723874	0.10
8	**"nd"**	-	-	-
9	**Vanillic acid**	9.639	14.54832	2.06
10	**Caffeic acid**	10.085	1.61106	0.23
11	**Syringic acid**	10.262	0.162395	0.02
12	**P- Coumaric acid**	13.041	0.234667	0.03
13	**Benzoic acid**	14.182	23.6418	3.35
14	**Ferulic acid**	15.454	0.498238	0.07
15	**Rutin**	16.53	22.888	3.24
16	**Ellagic**	17.052	6.39958	0.91
17	**O- Coumaric acid**	17.219	12.82712	1.82
18	**Resvertol**	19.751	71.52007	10.12
19	**Cinnamic acid**	20.05	0.801322	0.11
20	**Quercetin**	21.547	33.16893	4.69
21	**Rosemarinic**	22.05	5.29995	0.75
22	**Neringein**	22.291	6.60506	0.93
23	**Myricetin**	23.33	32.11736	4.54
24	**Kampherol**	23.926	6.36675	0.90
-	**Total**	-	706.662256	100

"nd" means "not detected"

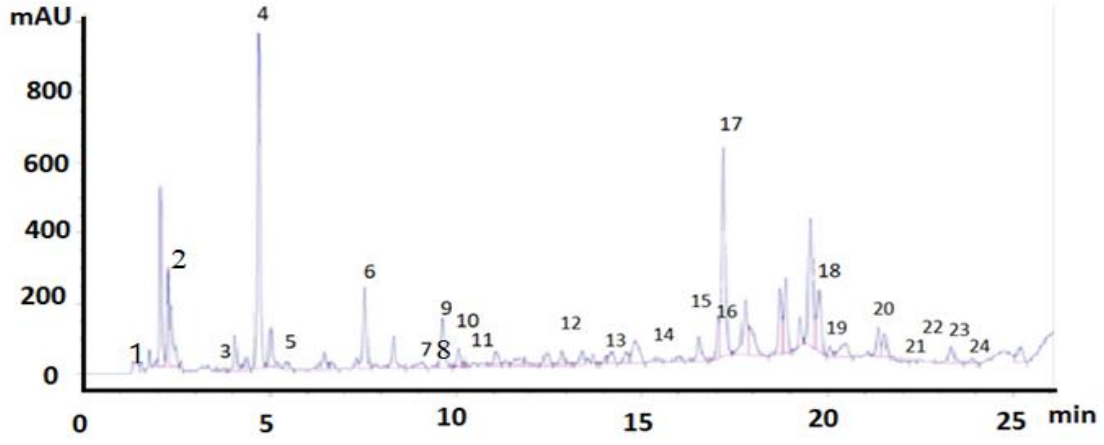
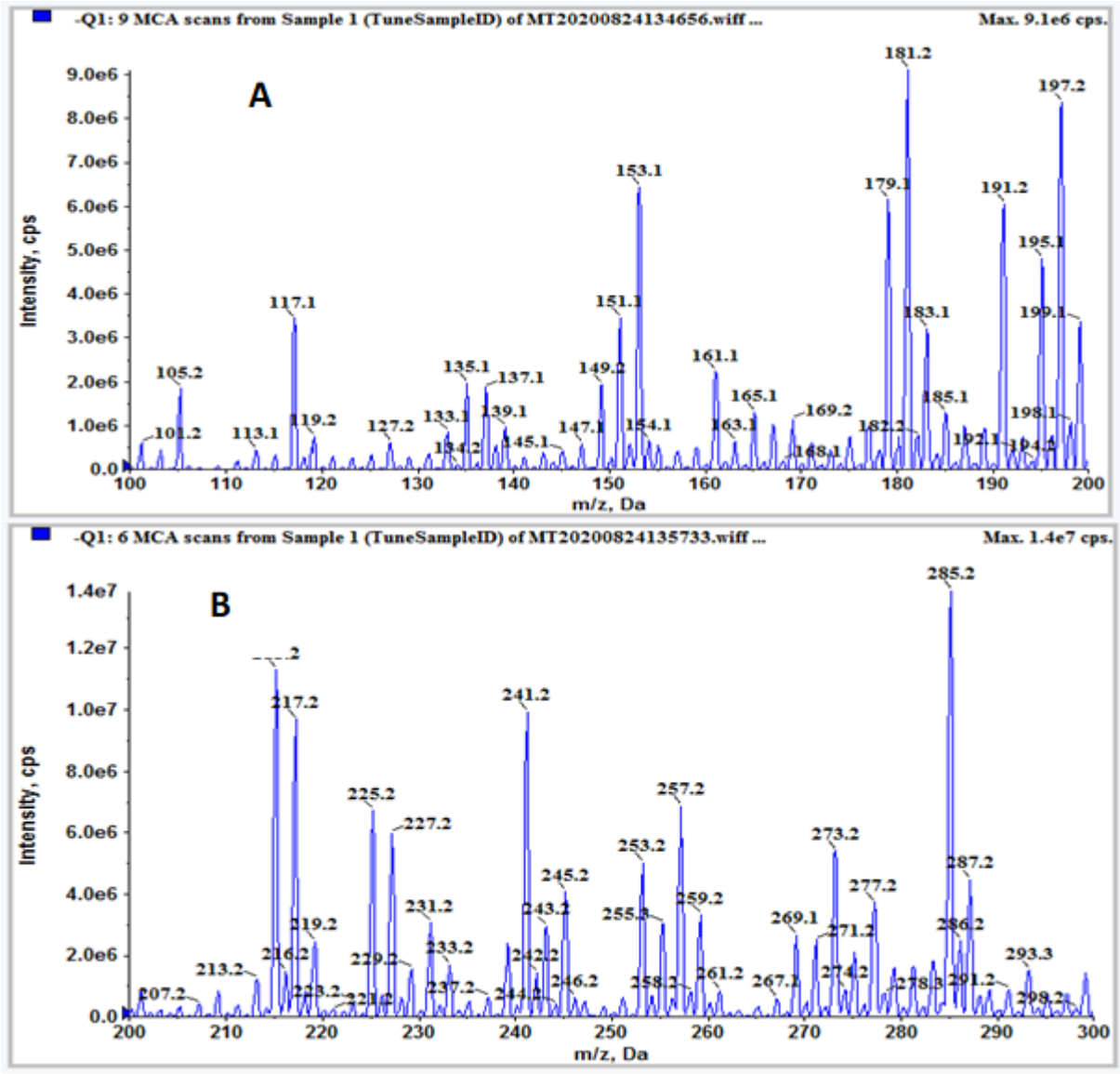


Figure 3. 3-Hydroxytyrosol (peak 4), O-coumaric acid (peak 17), Resvertol (peak 18), P-Hydroxybenzoic acid (peak 6), Vanillic acid (peak 9), Quercetin (peak 20), Syringic acid (peak 11), Myricetin (peak 23), Rutin (peak 15), Benzoic acid (peak 13), p-Coumaric acid (peak 12), Ellagic acid (peak 16), Ferulic acid (peak 14), Cinnamic acid (peak 19), Kampherol (peak 24), Neringein (peak 22), Rosemarinic acid (peak

21), Caffeic acid (peak 10), Catechol (peak 5), Catechin (peak 7), and Gallic acid (peak 3) were characterized by HPLC.

Table 2. Characterization of OLE phenolic compounds (antioxidants) by LC–MS/MS.

Number	Phenolic compounds identification	Molecular ion on first quadrupole (Q1)	Fragments ion on the third quadrupole (Q3)	Molecular formula
1	**Oleuropein**	539	(275-307)	$C_{25}H_{32}O_{13}$
2	**Luteolin-7-O-Glucoside**	447	285	$C_{21}H_{20}O_{11}$
3	**Apigenin-7-O-Glucoside**	431	268	$C_{21}H_{20}O_{10}$
4	**Quercetin**	301	-	$C_{15}H_{10}O_5$
5	**Luteolin**	285	133	$C_{15}H_{10}O_6$
6	**Apigenin**	269	(117-151)	$C_{15}H_9O_5$
7	**Homo vanilic acid**	181	-	$C_9H_{10}O_4$
8	**Caffeic acid**	179	135	$C_9H_8O_4$
9	**Hydroxytyrosol**	153	123	$C_8H_{10}O_3$
10	**Vanillin**	150.8	-	$C_8H_8O_3$
11	**Tyrosol**	137	-	$C_8H_{10}O$



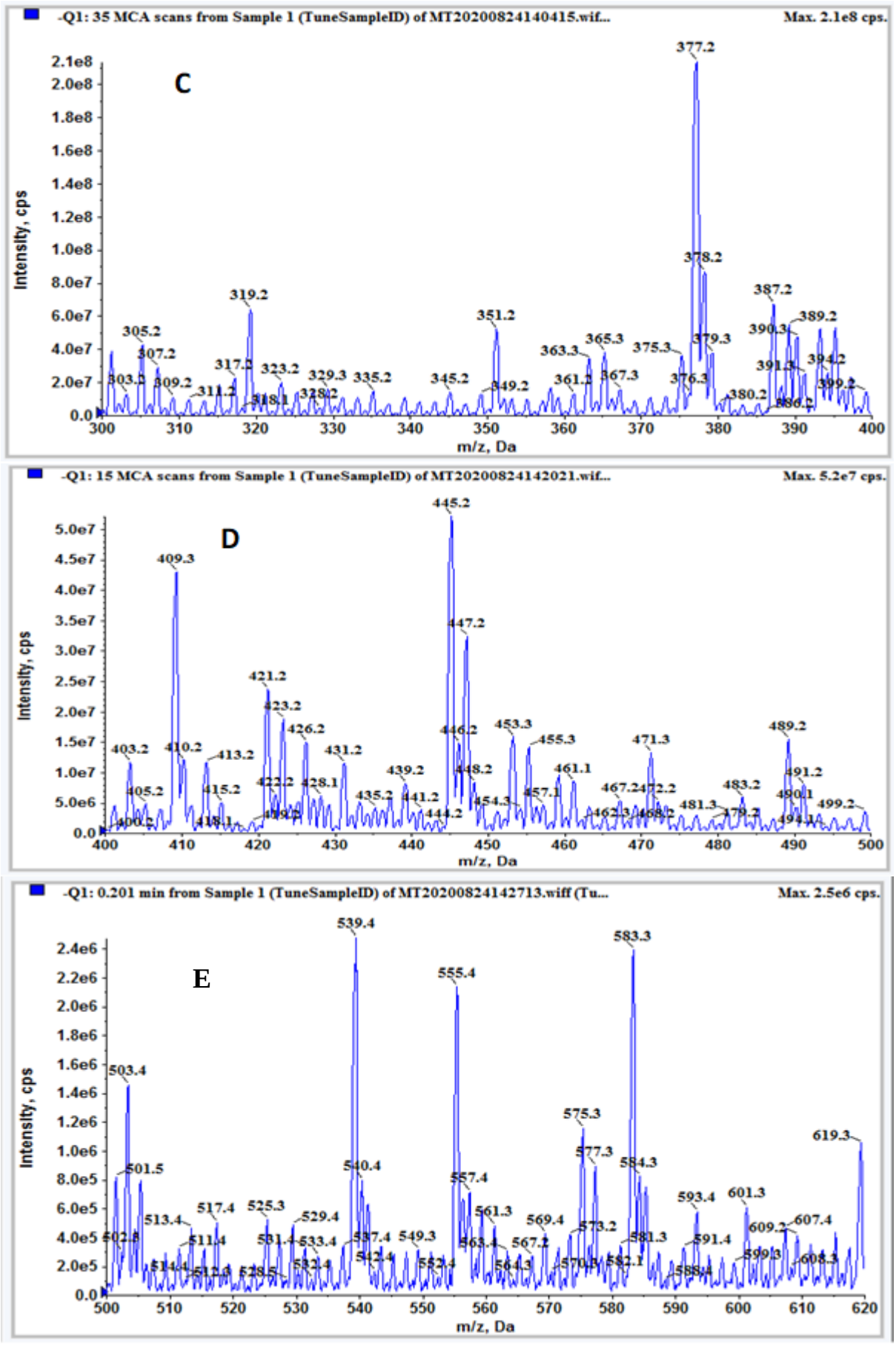


Figure 4. Characterization of total phenolic compounds from olive leaf extract (OLE) by LC–MS/MS. (A): 100:200 m/z. (B): from 200: 300 m/z, (C): from 300: 400 m/z, (D): from 400: 500 m/z, and (E): from 500: 620 m/z.

at m/z 431; an ion at m/z 301; an ion at m/z 285; an ion at m/z 285; an ion at m/z 285; an ion at m/z 269; a homo vanillic acid ion at m/z 181; an ion at m/z 179; a hydroxytyrosol ion at m/z 153; a vanillin ion at m/z 150.8; and a tyrosol ion at m/z 137.

The results of Sabry et al., (2014), who identified and measured luteolin 7-O-glucoside and oleuropein as the greatest phenolics in a lyophilized olive leaf extract, were consistent with our findings. Verbascoside, rutin, oleuropein, luteolin-7-o-glucoside, apigenin-7-o-glucoside, catechin, luteolin, vanillic acid, caffeic acid, hydroxytyrosol, and tyrosol were obtained from Mbarka Ben Mohamed et al., (2018). All of these findings demonstrated that OLE contains a substantial quantity of phenolic compounds that function as secondary metabolites.

4. CONCLUSIONS

Antioxidants are used in food processing, cosmetics, and pharmaceuticals, and olive leaves might be a good source of these substances. However, the bioactive components of olive leaves were investigated in this study. We outlined the profile of phenolic chemicals and concentrated on three extraction preparation techniques. To achieve a high phenolic content, olive leaves must be dried. This avoided any degradation of the phenolic compounds or olein content. Freeze-drying and air-drying are superior extraction techniques. Among the numerous identified phenolic compounds were 3-hydroxytyrosol, gallic acid, caffeic acid, catechin, P-hydroxybenzoic acid, syringic acid, rutin, catechol, P-coumaric acid, benzoic acid, fermentic acid, resvertol, elagic acid, cinnamic acid, o-coumaric acid, rosemarinic acid, vanillic acid, neringein, myricetin, quercetin, and kampherol. Nonetheless, bioactive compounds such as Oleuropein, Luteolin-7-O-glucoside, Apigenin-7-O-Glucoside, Quercetin, Luteolin, Apigenin, Homo vanillic acid, Caffeic acid, Hydroxytyrosol, Vanillin, and Tyrosol were identified by LC–MS/MS. Compared to fresh extracts, freeze-dried and air-dried extracts result in greater phenolic and flavonoid contents and increased antioxidant activity.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was funded by the Academy of Scientific Research and Technology (ASRT), Scientists for Next Generation program, Cycle 5 (SNG).

Author contributions

S.Z., A.A., M.A., M.H., and E.M. contributed to conceptualizing and planning the experiments. S.Z. carried out the experiments. S.Z., E.M. and M.H. contributed to the interpretation of the results. All authors contributed to the writing and revision of the manuscript.

Data availability

This published article contains all of the data collected or analysed during this investigation.

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الملخص العربي

تعريف وتوصيف المركبات الحيوية الفعالة في أوراق الزيتون

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مستخلص ورق الزيتون هو واحد من المستخلصات الطبيعية التي تُستخدم لإنتاج مركبات حيوية فعّالة. يحتوي على عدة مجموعات من المركبات الفينولية. تم تقييم المحتوى الكلي للفينولات والتركيز الكلي للفلافونويدات والنشاط المضاد للأكسدة من خلال استخدام طرق تجفيف مختلفة لأوراق الزيتون [التجفيف الهوائي (AD)، التجفيف بالتجميد (FD)، واستخدام أوراق الزيتون الطازجة (FE)] ، وتم تحديد أنواع مضادات الأكسدة باستخدام تحليل الكروماتوجرافي السائل. تم توصيف وتعريف مركبات فعّالة أخرى باستخدام تحليل كروماتوجرافي مزود بجهاز الكتلة للحصول على معلومات إضافية من المركبات المفصولة. أظهر المستخلص الإيثانولي (٨٠%) وجود محتوى عالٍ من الفينولات (التجفيف بالتجميد ١١٤,٥٥، التجفيف الهوائي ٩١,٩٥، والأوراق الطازجة ٦٣,٤١) مج/ج، بينما كان محتوى الفلافونويدات (٢٥١,٢٥ للتجفيف بالتجميد، ٢٥٤,٤٣ للتجفيف الهوائي، و١١١,٦٧ للأوراق الطازجة) مج/ج. تم استخدام DPPH لتقدير النشاط المضاد للأكسدة لمستخلص أوراق الزيتون (٨٢,٤٣ للتجفيف بالتجميد، ٨٩,٥٣ للتجفيف الهوائي، و٦٤,١٣ لمستخلص الأوراق الطازجة). وباستخدام تحليل الكروماتوجرافي السائل، تم توصيف وتعريف ٢١ مركبًا حيويًا فعّالًا. ومن بين المركبات الفينولية الهامة في مستخلص أوراق الزيتون التي تم تعريفها (الأوليوروبين، أبيجنين–٧–O–جليكوسيد، ليتولين–٧–O–جليكوسيد، ليتولين، كيرستين، وحمض الفينيلك، هيدروكسي تيروزول، حمض الكافييك، تيروزول، فانيلين). تشير نتائج هذه الدراسة إلى أن مضادات الأكسدة من المركبات الحيوية الفعّالة المستخرجة من أوراق الزيتون تُعتبر مصدرًا قيمًا ومهمًا، مما يجعلها من المواد التي يجب استخدامها في التطبيقات الصناعية العديدة، بما في ذلك مجالات الطب الدوائي والصناعات الغذائية.

الكلمات المفتاحية: ورق الزيتون، مضادات الاكسدة، المحتوى الكلي للفينولات، التحليل الكرومتوجرافي السائل