



Enhancement of Bioactive Compounds and Antioxidant Activities of Olive (*Olea europaea* L.) Leaf Extract by Instant Controlled Pressure Drop

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Abstract

In this work, the effect of Détente Instantanée Contrôlée (DIC) (French for instant controlled pressure drop) on the total polyphenol, flavonoids, α -tocopherol contents, and antioxidant activities of olive leaves was studied. Olive leaf extracts were pre-treated at one cycle DIC under 0.1 MPa pressure for 11 s and followed by an extraction with 95% ethanol at 55 °C during 3 h. The phenolic compounds, flavonoids, oleuropein, and α -tocopherol contents were determined, showing 66.63 mg gallic acid equivalent (GAE)/g db, 12 mg catechin equivalent (CE)/g db, 43.9 mg/g db, and 0.15 mg/g db for the untreated leaves against 239.37 mg GAE/g db, 28 mg CE/g db, 70.3 mg/g db, and 0.59 mg/g db for DIC-treated leaves, respectively. Therefore, DIC allows more availability of bioactive compounds contributing to a high antiradical activity (DPPH) compared to a synthetic antioxidant butylated hydroxytoluene (BHT). Both extracts showed a total antioxidant capacity (method of phosphomolybdenum) greater than that of the standard BHT. Likewise, both extracts have a reducing power (FRAP test) significant concentration-dependent. The DIC-treated leaves showed a higher antioxidant capacity compared to that of untreated leaves. Thus, DIC could be an effective treatment to promote the extraction of bioactive molecules of high antioxidant activities from olive leaves.

Keywords Olive leaves · Instant controlled pressure drop · Phenolic compounds · Antioxidant activities · Oleuropein · α -Tocopherol

Introduction

Lipid peroxidation is the major reason for the deterioration of food products during processing and storage. This process can be delayed by the addition of antioxidant compounds. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were widely used in foods and

cosmetics, but they have received restricted use as they are suspected to be highly toxic and carcinogenic (Lobo et al. 2010; Devi et al. 2015). Thus, the substitution of synthetic antioxidants by natural ones, especially of plant origin, has received a great interest in recent years. The natural antioxidant compounds may be ascorbic acid (vitamin C) and its salts (sodium ascorbate, calcium ascorbate, palmityl acid), tocopherols (α -, β -, γ -, and δ -tocopherols), and phenolic compounds. The phenolic compounds have been identified as free radical or active oxygen scavengers (Lobo et al. 2010; Tapas et al. 2008).

Olive leaves (*Olea europaea* L.) are one of the by-products of olive industries, which can be found in high amounts (10% of the total weight of the olives) (Boudhrioua et al. 2009; Ben Salah et al. 2012). In addition to this quantity, an important biomass is generated during the pruning of the olive trees.

Olive leaves were used in folk medicine for the treatment of hypertension, hypoglycemia, fevers, and other diseases (Benavente-Garcia et al. 2000; Mujić et al. 2011).

Olive leaves have been shown to be a source of valuable bioactive compounds (Souilem et al. 2017). The phenolic compounds identified in olive leaves are classified into secoiridoids,

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acids, and flavonoids (Benavente-Garcia et al. 2000). Indeed, the main phenolic compounds in olive leaves are oleuropein, hydroxytyrosol, tyrosol, and verbascoside (Benavente-Garcia et al. 2000; Rahmanian et al. 2015; Mkaouar et al. 2015; Souilem et al. 2017). Oleuropein is the major phenolic compound in olive leaf. Flavonoids constitute a large group of olive leaves (Heimler et al. 1992). They may be present as aglycone (quercetin, apigenin, luteolin, diosmetin) or in the glycosylated form (quercetin-7-O-rutinoside, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, luteolin-5-O-glucoside) (Ryan et al. 2002; Laguerre et al. 2009). Substituted phenols (hydroxytyrosol and tyrosol) are present in lower amounts than secoiridoids and flavonoids (Souilem et al. 2017). Tocopherols are present in olive leaves as α -tocopherol which is the predominant and most biologically active form (De Lucas et al. 2002).

Many studies have shown that olive leaf extract exhibits several activities, such as antioxidant (Ben Salah et al. 2012; Mujić et al. 2011; Lee and Lee 2010), antibacterial (Lee and Lee 2010; Pereira et al. 2007), and antiviral (Lee-Huang et al. 2003) activities. Furthermore, olive leaves possess hypotensive (Somova et al. 2003; Perrinjaquet-Moccetti et al. 2008), neuroprotective (Rabiei et al. 2012), and cardioprotective (Poudyal et al. 2010) properties.

It is known that olive leaf extract exhibits a synergic behavior, characteristic of its content of oleuropein and other phenolic compounds (Benavente-Garcia et al. 2000; Lee and Lee 2010).

The intensification of the olive leaf phenolic compounds extraction was studied through different processes such as ultrasound-assisted extraction (Japón-Luján et al. 2006), sub-critical fluid extraction (Le Floch et al. 1998; Xynos et al. 2012), superheated liquid extraction (Japón-Luján and Luque de Castro 2006), microwave-assisted extraction (Şahin et al. 2017), and instant controlled pressure drop (DIC) pretreatment-assisted extraction (Mkaouar et al. 2015).

The DIC treatment was reported as an interesting process to intensify the conventional extraction, since it improves the solvent extraction and generates extracts greatly rich in bioactive compounds (Mkaouar et al. 2016; Namir et al. 2017). The optimum conditions for the DIC treatment prior to the extraction of olive leaf polyphenols were studied in a previous work (Mkaouar et al. 2015). In this way, the present study was carried out to investigate the impact of DIC treatment on the extraction of bioactive compounds of olive leaf. Total phenolic compounds, total flavonoids and α -tocopherols, and the antioxidant activities of the olive leaf extracts were evaluated.

Materials and Methods

Raw Materials

Olive leaves (*Olea europaea* L.) from Chemlali variety were collected from the arboretum of the National Engineering

School of Sfax, Tunisia, in May 2016, cleaned with distilled water, and dried in an airflow oven at 40 °C. The dried material was ground using a grinder (Retsch Grindomix, GM 200) at 10,000 rpm for 1 min.

Chemicals

Chemicals, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxyanisole (BHT), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium hydroxide, and other solvents, were of analytical grade. The DL- α -tocopherol was purchased from Merck (France). Oleuropein, hydroxytyrosol, tyrosol, vanillin, vanillic acid, apigenin-7-glucoside, luteolin-7-glucoside, and verbascoside were obtained from Sigma-Aldrich (Steinheim, Germany).

DIC Treatment

The DIC treatment of olive leaves was previously described by Mkaouar et al. (2015). Briefly, fresh olive leaves were placed in the DIC treatment vessel under vacuum. Then, a thermal treatment (high temperature—short time) stage is established with high-pressure saturated steam ranging from 0.1 to 0.7 MPa. After that, the pressure undergoes an abrupt drop towards a vacuum, which induced an instant autovaporization resulting in an “instant” cooling of the solid material. The DIC treatment conditions were optimized in a previous study (Mkaouar et al. 2015). These conditions are pressure $P = 0.1$ MPa, number of cycles $C = 1$, and total treatment time $t = 11$ s.

DIC-treated olive leaves were immediately dried in an oven at 40 °C and then ground to powder using a grinder (Retsch Grindomix, GM 200) at 10,000 rpm for 1 min.

Extraction Process

The extracts were prepared using 1 g of olive leaf powder suspended in 25 ml of 95% EtOH concentration at 55 °C for 3 h for untreated and DIC-textured olive leaves ($P = 0.1$ MPa; number of cycles = 1; total time = 22 s). After the extraction procedure, both extracts were filtered with a filter paper. The filtrates were recovered in 4 °C until the time of analysis.

Determination of Total Phenolic Content

Total phenolic compound contents (TPC) of the untreated and DIC-treated olive leaf extracts were determined according to the Folin-Ciocalteu method (Škerget et al. 2005).

Gallic acid in ethanol was used as a standard. The results were expressed in milligram of gallic acid equivalent per gram of dry matter of olive leaves (mg GAE/g db).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined by using a colorimetric method described by Dewanto et al. (2002). Briefly, an aliquot of 0.25 ml of diluted olive leaf extracts were added to 75 μ l of a 5% NaNO₂ solution. The sample was incubated for 6 min, and then 150 μ l of a 10% AlCl₃ solution was added. After 5 min, 0.5 ml of 1 M NaOH was added. The mixture was brought to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using an UV mini 1240, UV/VIS spectrophotometer (SHIMADZU, China). Catechin was used as a standard. The results were expressed in milligram of catechin equivalent per gram of dry basis of olive leaves (mg CE/g db).

UPLC Analyses

The content of phenolic compounds was determined using ultra-performance liquid chromatography (Waters Instruments, Acquity UPLC H-CLASS, Singapore) coupled to a diode array detector (PDA Waters UPLC LG 500 nm) according to the protocol previously described (Mkaouar et al. 2015; Mkaouar et al. 2016).

Determination of Tocopherols and Tocotrienols

The contents of tocopherols were determined by high-performance liquid chromatography (HPLC) (Shimadzu, Japan), coupled with fluorimetric detection (excitation, 290 nm; and emission, 330 nm) according to the method of ISO 9936 (2006 F). The column used is a silica column (Lichrospher 100 diol, 5 mm). The flow rate was 1 ml/min and the column temperature was maintained at 25 °C. The elution solvent is a tetrahydrofuran solution in n-heptane (3.85%). Tocopherols and tocotrienols were quantified based on the external standards.

DPPH Radical Scavenging Activity

The free radical scavenging activity was measured in terms of radical scavenging ability by using the free radical DPPH (100 μ M) in ethanol. The DPPH radical scavenging capacity of olive leaf extracts was determined as described by Ben Mansour et al. (2013). A volume of 500 μ l of each sample prepared at different concentrations (25–750 μ g/ml) was added to 375 μ l of absolute ethanol and 125 μ l of DPPH solution (0.02% in ethanol) as a free radical source. The mixtures were shaken and then incubated for 60 min in the dark at room temperature. Radical scavenging capacity was measured using a spectrophotometer UV mini 1240, UV/VIS (SHIMADZU, China), by monitoring the decrease in

absorbance at 517 nm. DPPH radical scavenging capacity was calculated as follows:

DPPH radical scavenging activity (%)

$$= \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control solution (containing all reagents except the sample) and A_{sample} is the absorbance of the sample.

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Butylated hydroxytoluene (BHT) was used as a positive control.

Ferric Reducing Antioxidant Power Assay

The ability of the extracts to reduce Fe³⁺ was determined according to the method of Yildirim et al. (2001) with slight modifications. An aliquot of 500 μ l of each sample prepared at different concentrations (25–200 μ g/ml) was mixed with 1.25 ml of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 30 min, followed by addition of 1.25 ml of 10% (w/v) trichloroacetic acid. A volume of 1.25 ml of this solution was mixed with 1.25 ml of distilled water and 250 μ l of 0.1% ferric chloride (w/v). After 10 min of reaction, the absorbance of the solution was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Determination of Total Antioxidant Capacity by Ammonium Molybdate Reduction Method

This method is based on the reduction of Mo (VI) to Mo (V) by the formation of a green phosphate/Mo(V) complex at acidic pH. Total antioxidant capacity was measured according to the method of Prieto et al. (1999). An aliquot of 100 μ l of each extract prepared at different concentrations (10–150 μ g/ml) was mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After 90 min of incubation at 95 °C, samples were cooled to room temperature and the absorbance of molybdate (V) was measured at 695 nm using an UV mini 1240, UV/VIS spectrophotometer (SHIMADZU, China). The antioxidant activity was expressed as the number of equivalents of α -tocopherols (μ mol/ml).

Statistical Analysis

All measurements were carried out in triplicate. The results are expressed as mean values \pm standard deviations. The data were statistically analyzed using the Statistical Package for

Social Sciences (SPSS) version 20.0. The *T* test for paired samples was used to determine the effect of the treatment on different parameters. Statistical significance was accepted at a level of $p < 0.05$.

Results and Discussion

Antioxidant Contents

Total phenolics (TPC), total flavonoids (TFC), phenolic compounds, and α -tocopherol contents of untreated and DIC-treated olive leaf extracts are given in Table 1.

Total Phenolic Content

Total phenolic compounds in untreated and DIC-treated olive leaf extracts were determined according to the Folin-Ciocalteu assay. As shown in Table 1, the total polyphenol content in untreated leaves was 66.63 mg GAE/g db. After DIC treatment, there was a significant ($p < 0.05$) improvement of the polyphenol content. In fact, TPC amount increases from 66.63 to 239.37 mg GAE/g db for DIC-treated leaves. Thus, DIC is an effective treatment for the intensification of phenolic compounds of olive leaves. This can be attributed to the thermomechanical effects of the DIC treatment, which improves the availability of active molecules in olive leaves (Mkaouar et al. 2015).

Different extraction methods were used to evaluate the phenolic compounds in olive leaves (Souilem et al. 2017). The TPC (239 mg GAE/g db) obtained in this study is the highest in comparison to that obtained in other studies. In fact, pressurized liquid extraction allows to have total polyphenol yield of 58.70 mg/g db (Herrero et al. 2011). Likewise, total

polyphenol content obtained with ultrasound-assisted extraction varies from 25.14 (Japón-Luján et al. 2006) to 45.8 mg/g db (Ahmad-Qasem et al. 2013). In addition to the method of extraction, the variety of olive leaves, the nature of the solvent, and process time could lead to different yields of phenolic compounds (Ben Salah et al. 2012; Rafiee et al. 2012).

Oleuropein is the major phenolic compound of untreated DIC-treated olive leaves. Oleuropein content is greatly improved after DIC treatment (70.3 mg/g db) in comparison with that of untreated leaf extract (43.9 mg/g db) (Table 1). Oleuropein is the major phenolic compound of untreated and DIC-treated olive leaf extracts followed by verbascoside, luteolin-7-glucoside, hydroxytyrosol, apigenin-7-glucoside, tyrosol, vanillic acid, and vanillin.

In fact, DIC treatment showed a positive impact on the extraction of different molecules such as flavonoids (Ben Amor and Allaf 2009), essential oils (Berka-Zougali et al. 2010; Besombes et al. 2010), oligosaccharides (Amor et al. 2008), and antioxidants (Allaf et al. 2013; Namir et al. 2017).

Total Flavonoid Content

Flavonoids represent the most common and widely distributed group of phenolic compounds (more than 4000 flavonoids have been identified) (Ignat et al. 2011). They are ubiquitous in fruits, vegetables, seeds, and beverages (Tsimogiannis and Oreopoulou 2006). Flavonoids are particularly important antioxidants because of their high redox potential which allows to act as reducing agents, hydrogen donors, and scavengers of singlet oxygen (Tsao and Yang 2003).

In this study, the amount of total flavonoids in untreated leaf extract is 12 mg CE/g MS. This result is in accordance with that found by Botsoglou et al. (2012) for Greek olive leaves (12.46 mg CE/g db), but lower than that reported by Makris et al. (2007) with a content of 16.78 mg catechin equivalent (mg CE/g db). These differences may be due to the method of extraction, the variety of olive leaves, and the solvent type.

After DIC treatment, TFC amount increased significantly ($p < 0.05$) to reach a value of 28 mg CE/g MS (about two times higher than that of raw material). This could be explained by the impact of the DIC treatment on the microstructure of olive leaves which makes solvent extraction more easier and bioactive molecules more available (Mkaouar et al. 2015).

Tocopherols Contents

Tocopherols were detected and quantified by high-performance liquid chromatography (HPLC) according to the method of ISO 9936 (2006 F). This method is specific for the determination of levels of α -, β -, γ -, and δ -tocopherols and tocotrienols (tocols) in fats and oils (fats) from animal and vegetable origin.

Table 1 Total phenolics, flavonoids, and α -tocopherol contents of untreated and DIC-treated olive leaves

Compound	Untreated leaves	DIC-treated leaves
TPC (mg GAE/g db)	66.63 \pm 0.01 ^a	239.37 \pm 0.25 ^b
TFC (mg CE/g db)	12.00 \pm 0.02 ^a	28.35 \pm 0.05 ^b
Hydroxytyrosol	0.39 \pm 0.02 ^a	0.51 \pm 0.02 ^b
Tyrosol	0.3 \pm 0.01 ^a	0.4 \pm 0.03 ^a
Acide vanillique	0.3 \pm 0.01 ^a	0.3 \pm 0.02 ^a
Vanilline	0.1 \pm 0.02 ^a	0.1 \pm 0.03 ^a
Lutéoline-7-glucoside	0.92 \pm 0.03 ^a	1.2 \pm 0.01 ^a
Verbascoside	5.5 \pm 0.02 ^a	6.55 \pm 0.02 ^b
Apigénine-7-glucoside	0.49 \pm 0.02 ^a	0.51 \pm 0.02 ^a
Oleuropein (mg/g db)	43.9 \pm 0.05 ^a	70.3 \pm 0.02 ^b
α -tocopherol (mg/g db)	0.15 \pm 0.01 ^a	0.59 \pm 0.02 ^b

Means not sharing the same letter in the same line are significantly different ($p < 0.05$)

HPLC analysis of the heptanoic extracts of olive leaves shows that the major compound is the α -tocopherol. The β -, γ -, and δ -tocopherols are present in trace amounts. The α -tocopherol content of untreated leaves is 0.15 mg/g db (Table 1). This result is in agreement with that of Mohamed et al. (2007) who found that total tocopherol content of olive leaves was equal to 0.136 mg/g. The α -tocopherol was the major compound, which accounts for 0.133 mg/g. The contents of β -, γ -, and δ -tocopherols were equal to 0.001 mg/g each.

It is also noted that α -tocopherol content (0.58 mg/g db) of DIC-treated leaves is significantly higher ($p < 0.05$) in comparison with that of untreated leaves (Table 1). This value is higher than that found by De Lucas et al. (2002) who studied the extraction of tocopherols from olive leaves using supercritical fluid extraction. Indeed, the content of α -tocopherol varies from 0.06 to 0.1 mg/g leaf depending on different conditions of supercritical fluid extraction.

The DIC treatment improved the extraction of α -tocopherol. This could be explained by the significant mechanical impact of the instantaneous pressure drop on the olive leaf that promotes solvent extraction and availability of active molecules.

Tocopherols are present in olive leaves in the form of α -tocopherol which is the predominant and most biologically active form (De Lucas et al. 2002). Tocopherols play the role of antioxidants, acting to prevent the peroxidation of unsaturated lipids by free radicals. They can also play a role in stabilizing the structure of cell membranes and modeling the immune response in chains (Hall 2001).

Thus, olive leaves could be considered as a natural source rich in vitamin E which acts as an antioxidant.

DPPH Radical Scavenging Activity

The antioxidant capacity of untreated and DIC-treated olive leaf extracts was measured in terms of radical scavenging ability, using the stable radical, DPPH reagent at different concentrations (25–750 $\mu\text{g/ml}$). BHT was used as positive control. The reduction of DPPH is followed by monitoring the decrease in its absorbance at 517 nm during the reaction. The results of the DPPH radical scavenging activity are presented in Fig. 1.

As shown in Fig. 1, the DPPH scavenging capacities of untreated and DIC-treated leaf extracts increased with concentrations. DIC-treated leaf extract presented the highest radical scavenging ability (95.7%) at a concentration of 750 $\mu\text{g/ml}$, followed by untreated leaf extracts (94.5%) and BHT 88.4%. Therefore, olive leaf extracts may possess a strong DPPH radical scavenging ability at relatively low concentrations. Their radical scavenging activities were significantly higher than those of BHT. These results are in accordance with those of Namir et al. (2017) who reported that DPPH radical

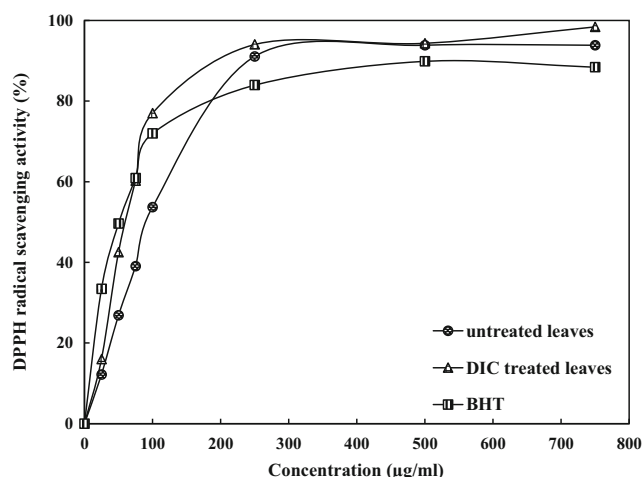


Fig. 1 Radical scavenging activity (on the radical 1,1-diphenyl-1-picrylhydrazyl) of untreated and DIC-treated olive leaf extracts and BHT

scavenging activity of cactus pear peel was increased up to 53% after DIC treatment.

The strong DPPH scavenging activity of olive leaf extracts could be attributed to their high total polyphenolic content (Mkaouar et al. 2015). These results are in agreement with several studies which showed that individual and combined phenolic compounds of olive leaf extracts exhibited higher radical scavenging activities than control antioxidants (BHT and ascorbic acid) (Lee and Lee 2010; Hayes et al. 2011; Ben Salah et al. 2012). Indeed, phenolic compounds in olive leaf extracts exhibited high antiradical activity (Moudache et al. 2016).

Benavente-Garcia et al. (2000) demonstrated that the scavenging activity of olive leaf is due to their phenolic compounds with high synergistic effect. El Sedef and Karakaya (2009) revealed that hydroxytyrosol, oleuropein, caffeic acid, and tyrosol were able to prevent the generation of reactive oxygen species by intact leukocytes, without evidence of toxicity. Oleuropein and its metabolite hydroxytyrosol have a catechol group necessary for antioxidant scavenging activity. These two compounds have been reported as scavengers of superoxide anion and efficient scavengers of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Ben Salah et al. (2012) showed also that olive leaf extracts exhibit higher radical scavenging activity than BHT and ascorbic acid.

The inhibitory concentration (IC_{50}) values of the different extracts were also determined and are presented in Table 2. In fact, the lower the IC_{50} value, the higher the radical scavenging activity on DPPH. The IC_{50} of the untreated leaves (86.88 mg/ml) is higher ($p < 0.05$) than that of BHT (50.88 mg/ml). The IC_{50} value of the DIC-treated leaf extract (60.17 mg/ml) is lower than that of the untreated leaves, but remains higher than that of the BHT. These results show that the DIC treatment permits to have higher DPPH radical scavenging activity of olive leaf extracts.

Table 2 IC₅₀ values of untreated and DIC-treated olive leaf extracts and BHT

Extract	Untreated leaves	DIC-treated leaves	BHT
IC ₅₀	86.88 ± 3.22 ^a	60.17 ± 2.57 ^b	50.88 ± 4.75 ^c

Means not sharing the same letter in the same line are significantly different ($p < 0.05$)

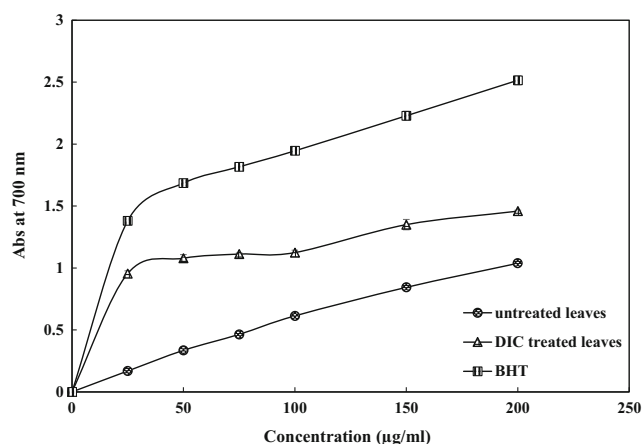
Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power assay (FRAP) assay is based on the measurement of the ability of the substance to reduce ferricyanide complex (Fe^{3+}) to ferrous form (Fe^{2+}). The presence of reductones is responsible for this reducing power (Xing et al. 2005). In fact, the reductones exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. Thus, the FRAP assay could be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al. 2002). So, a higher absorbance at 700 nm indicates a higher reducing power.

Figure 2 shows the reducing power of untreated and DIC-treated olive leaf extracts as a function of their concentrations. BHT was used as a positive control.

Untreated and DIC-treated olive leaf extracts exhibited a lower reducing power effect than BHT. In fact, at 200 $\mu\text{g}/\text{ml}$, reducing powers of untreated and DIC-treated leaves were 1.038 and 1.514, respectively. The reducing power of BHT was 2.515. These results are in agreement with several studies, which revealed that olive leaf extract exhibited moderate reducing power effects (Ferreira et al. 2007; Rafiee et al. 2012).

The DIC treatment improves the reducing power of olive leaves. This could be explained by the improvement of polyphenols extraction by DIC treatment which generates extracts greatly rich in bioactive compounds, responsible factors for the antioxidant activity (Mkaouer et al. 2015).

**Fig. 2** Reducing power of hydro-ethanolic extracts of untreated and DIC-treated olive leaves and BHT

The antioxidant activity of the phenolic compounds in the olive leaf extract could be due to the presence of hydroxyl groups in their structure such as oleuropein, hydroxytyrosol, and luteolin-7-O-glucoside (Visioli et al. 2002; Visioli and Galli 2002). Thus, the presence of reducing agents in the extract such as glycosylated phenolic compounds causes the conversion of complex ferricyanide Fe^{3+} to Fe^{2+} , knowing that the iron acts as a reactive metal, which catalyzes oxidative damage in living tissues and cells (Bourgou et al. 2008).

The abundance of phenolic compounds present in olive leaf extracts has the ability to donate electrons to the reactive free radicals, converting them into stable components and terminating the free radical chain reaction.

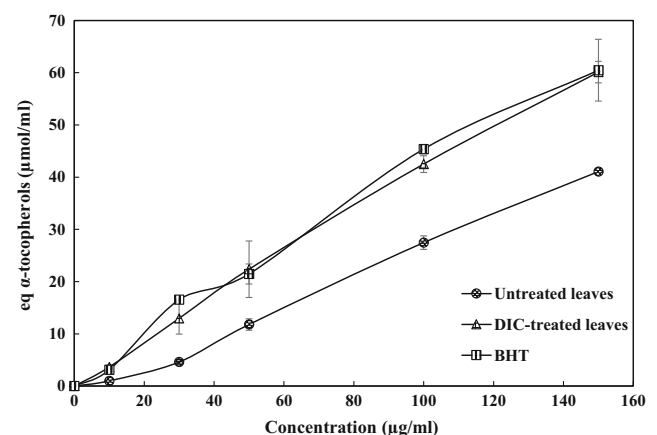
The study of Rafiee et al. (2012) also showed that all extracts of olive leaves obtained after extraction by microwave had low ferric reducing power compared to BHT.

Total Antioxidant Activity

The reduction of ammonium molybdate by untreated and DIC-treated olive leaf extracts and BHT are presented in Fig. 3.

Figure 3 shows that the total antioxidant activity of hydro-ethanolic extracts of untreated and DIC-treated olive leaves increased with increasing concentration of the extracts. The total antioxidant activity of DIC-treated leaf extract is higher than that of BHT, while the untreated leaf extract has a lower antioxidant activity compared with that of BHT and DIC-treated leaf extract. In fact, at a concentration of 150 $\mu\text{g}/\text{ml}$, total antioxidant capacity of untreated and DIC-treated leaves and BHT was 41.05 and 69.24 and 60.46 $\mu\text{mol } \alpha\text{-tocopherols}/\text{ml}$, respectively.

DIC treatment showed a great impact on the improvement of the total antioxidant activity of olive leaf extract. This could be explained by the improvement of the olive leaf polyphenol extraction.

**Fig. 3** Total antioxidant activity of hydro-ethanolic extracts of untreated and DIC-treated olive leaves and BHT

Conclusion

In the present study, the impact of DIC treatment on the antioxidant compounds (flavonoids, oleuropein, and tocopherols) of olive leaves and their antioxidant activities was evaluated. The DIC treatment improved the extraction of antioxidant compounds and the antioxidant activities of olive leaf extract. In fact, DIC-treated leaf extract was found to possess higher amounts of total phenolic compounds, flavonoids, and alpha-tocopherol as well as better antioxidant activities in comparison with the untreated leaf extract. Therefore, the olive leaves treated by DIC could be used as potential antioxidant agents in biological systems due to their high levels of hydrogen-donating antioxidant compounds and their abilities to scavenge oxygen species.

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