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Chétoui olive leaf extracts: influence of the solvent type on phenolics and antioxidant activities

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RESUMEN

Extractos de hojas de olivo de Chétoui: Influencia del tipo de disolventes sobre los compuestos fenólicos y su actividad antioxidante.

El objetivo de este trabajo fue estudiar la influencia del disolvente usado en la extracción de los compuestos fenólicos y en las propiedades antioxidantes de los extractos obtenidos a partir de hojas de olivo procedente de la variedad Chétoui. La extracción se realizó a temperatura ambiente, usando cuatro disolventes: agua desionizada (ddH₂O), metanol 80% (80% MeOH), etanol 70% (70% EtOH), y acetona 80%. Los fenoles totales y los flavonoides totales se midieron usando los métodos colorimétricos con Folin-Ciocalteu y cloruro de aluminio, respectivamente. Las propiedades antioxidantes han sido determinadas por dos métodos: el DPPH y el ABTS. El contenido de fenoles totales y flavonoides de los extractos de hojas de olivo (OLEs) varió desde 16,52 hasta 24,93 mg de ácido gálico g⁻¹ de materia seca y desde 6,23 hasta 21,47 mg de catequina g⁻¹ de materia seca, respectivamente. Los valores de IC₅₀ de DPPH varían de 0,17 a 0,97 mg mL⁻¹ y los valores de la actividad antioxidante determinados por ABTS (capacidad antioxidante en equivalentes de trolox) se encuentran entre 629,87 y 1064,25 μmol TE g⁻¹ de materia seca, respectivamente. Nuestros resultados revelaron que los disolventes de extracción tienen una influencia significativa en las propiedades antioxidantes de los extractos de hojas de olivo. El metanol se recomienda para los extractos con alto nivel de flavonoides y con actividades antioxidantes importantes. Además, se observó que la capacidad antioxidante en equivalente de trolox (TEAC) depende más de los flavonoides ($r = 0,821$) que de los fenoles totales ($r = 0,399$).

PALABRAS CLAVE: Actividades antioxidantes – Disolvente de extracción – Fenoles – Flavonoides – Hojas de olivo.

SUMMARY

Chétoui olive leaf extracts: influence of the solvent type on the phenolics and antioxidant activities.

The aim of this study was to investigate the influence of the solvent type on the extraction of phenolics and the antioxidant properties of the extracts obtained from Chétoui olive leaves. Extraction was conducted at room temperature using four solvents: deionized water (ddH₂O), 80% methanol (80% MeOH), 70% ethanol (70% EtOH), and 80% acetone. Total phenols and total flavonoids were measured using the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. The antioxidant properties have been determined by two

scavenging activity methods, DPPH and ABTS. The total phenol and flavonoid contents of the olive leaf extracts (OLEs) ranged from 16.52 to 24.93 mg gallic acid g⁻¹ DM and from 6.23 to 21.47 mg catechin g⁻¹ DM, respectively. The IC₅₀ values of DPPH varied from 0.17 to 0.97 mg mL⁻¹ and the antioxidant activity values determined by ABTS (trolox equivalent antioxidant capacity) were between 629.87 and 1064.25 μmol TE g⁻¹ DM. Our results revealed that extracting solvents have a significant influence on the antioxidant properties of olive leaves and that a methanol mixture is recommended for extracts with high levels of flavonoids and important antioxidant activities. Moreover, it was noticed that the trolox equivalent antioxidant capacity (TEAC) depends more on the flavonoids ($r = 0.821$) than on the total phenols ($r = 0.399$).

KEY-WORDS: Antioxidant activities – Flavonoids – Olive leaves – Phenolics – Solvent extraction.

1. INTRODUCTION

The Chétoui variety is the second main variety cultivated in the north of Tunisia. It covers an area of 176,000 Ha and accounts for more than 20% of the olive oil produced in Tunisia. It shows a high capacity of adaptation to various pedoclimatic conditions and is a vigorous cultivar. The fruits have dual use as table olives and for oil. The lipid yield is about 20-30%. The oil is characterized by important levels of phenols, o-diphenols, tocopherols and good resistance to oxidation; its sensorial characteristics are much appreciated by consumers (Manai *et al.*, 2007; Ben Temime *et al.*, 2008). Hence, a complete characterization and valorization of products and derivatives of this cultivar are currently one of our interests.

While olive oil is well known for its flavor and health benefits, the olive leaf is traditionally associated with a wide number of medicinal claims. Epidemical studies revealed the antimicrobial, antifungal, antiinflammatory and antiviral effects of olive leaf extracts (OLEs) (Bisignano *et al.*, 1999; Furneri *et al.*, 2002; Micol *et al.*, 2005). In the same context, we demonstrated that apigenin 7-glucoside from olive leaf extracts of the Tunisian Gerbouli cultivar induced the differentiation of the human Leukemia HL-60 cells (Abaza *et al.*, 2007).

Studies show that the health benefits of OLEs are linked to its antioxidant properties due to the bioavailability of some phenolic compounds and their derivatives such as oleuropein, verbascoside, ligstroside, tyrosol and hydroxytyrosol, etc. (Garcia *et al.*, 2000; Savournin *et al.*, 2001; Meirinhos *et al.*, 2005). It was found that enrichment with olive leaf extracts is sufficient to fortify refined oil to a similar stability as a virgin olive oil (Paiva-Martins *et al.*, 2007). Likewise, it was reported that phenolic extracts obtained from the olive plant (fruit, leaves and pomace) showed remarkable antioxidant activity in retarding sunflower oil oxidative rancidity (Farag *et al.*, 2003). Therefore, olive leaves can be considered a cheap raw material and a safe and natural potential of antioxidants.

There are many methods used for extracting phenolics from their plant sources and studies show that no single extraction protocol can be considered optimal for all types of samples. In fact, many factors influence the efficiency of solvent extraction, such as the type of solvent, pH, temperature, number of steps and volume of solvent along with particle size in the sample (Escribano and Santos-Buelga, 2003).

Alcoholic solvents have been commonly employed to extract phenolics from natural sources (Spigno *et al.*, 2007). The most widely used is methanol and methanol/water mixtures. Soluble phenolic compounds are generally extracted using water, methanol, ethanol or acetone. The presence of attached sugar tends to render the phenolic compounds more water soluble, and the combination of the above solvents with water are thus better solvents for glycosides. In contrast, less polar aglycones such as isoflavones, flavanones and highly methoxylated flavonols tend to be more soluble in non aqueous solvents (Escribano and Santos-Buelga, 2003).

Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts (Calliste *et al.*, 2005).

The aim of the work was to study the influence of the solvent type on the efficiency of the extraction of total phenols and flavonoids and the antioxidant properties of extracts obtained from Tunisian Chétoui olive leaves.

2. MATERIALS AND METHODS

2.1. Chemicals

The methanol, acetone, ethanol, sodium carbonate, sodium nitrite, sodium hydroxide, potassium peroxodisulphate and Folin-Ciocalteu were purchased from Fluka (Buchs, Switzerland). The 2, 2' -Azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2, 2 diphenyl -1- picrylhydrazyl (DPPH) were from Sigma Co. (St. Louis, MO, USA). The 6, hydroxy-2,5,7,8 tetramethyl chroman 2, carboxylic acid (trolox) was from Aldrich. Gallic acid, (+)-catechin, and aluminium chloride were obtained from Sigma-Aldrich (Steinheim, Germany).

2.2. Extract preparation

Four solvents were used for extracting phenolics from leaves: 80% methanol (80% MeOH), 70% ethanol (70% EtOH), 80% acetone and deionized water (ddH₂O).

Fresh olive leaves of the Chétoui variety were obtained from an orchard located in the "Center of Biotechnology of Borj-Cédria". One gram of leaves was ground with a mortar and pestle under liquid nitrogen and then 10 mL of solvent were added. The mixture was allowed to stand in the dark for 24 h. The extract was centrifuged at 5 000 g for 10 min, at room temperature, and the supernatants were then filtered using a filter paper.

2.3. Total phenolic content (Folin–Ciocalteu assay)

Total phenolics were determined using the Folin–Ciocalteu reagent method (Skerget *et al.*, 2005) with minor modifications. To 0.5 mL of diluted extract, 2.5 mL of Folin–Ciocalteu reagent (diluted 10-fold with ddH₂O) were added and allowed to stand at room temperature for 3mn. Then 2 mL of Na₂CO₃ (75 g L⁻¹) were added. The sample was incubated for 5 min at 50 °C and then cooled. For a control sample, 0.5 mL of ddH₂O was used. The absorbance was measured at 760 nm. The results were expressed as mg of gallic acid equivalents per g of dry (DM) or fresh matter (FM) (mg GAE g⁻¹ DM or mg GAE g⁻¹ FM).

2.4. Total flavonoids

The determination of flavonoids was performed according to the colorimetric assay of Kim *et al.* (2003). ddH₂O (4 mL) was added to 1 mL of olive leaf extract. Then, a 5% sodium nitrite solution (0.3 mL) was added, followed by a 10% aluminum chloride solution (0.3 mL). Test tubes were incubated at ambient temperature for 5 min, and then 2 mL of 1 M sodium hydroxide were added to the mixture. Immediately, the volume was made to 10 mL with ddH₂O. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. The amount of total flavonoids was expressed as mg catechin equivalents per g of dry or fresh matter (mg CE g⁻¹ DM or mg CE g⁻¹ FM).

2.5. DPPH assay

The DPPH radical scavenging activity was evaluated according to the slightly modified procedure described by Brand-Williams *et al.* (1995). An aliquot of olive leaf extract (0.1 mL) at different concentrations was added to 3.9 mL of a DPPH solution (6 x10⁻⁵ M in methanol). The reaction mixture was then shaken vigorously and left to stand in the dark at room temperature for 30 min. The ability to scavenge DPPH radicals was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]}{[\text{Abs}_{\text{control}}]} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical + solvent; $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample extract.

The IC₅₀ values denote the concentration in (mg mL^{-1}) of sample which is required to scavenge 50% of DPPH free radicals.

2.6. ABTS assay

The ABTS radical (ABTS^+) scavenging assay was carried out using the improved ABTS method, as described by Tawaha *et al.* (2007). Briefly, ABTS^+ radical is generated by reacting 7 mM ABTS and 2.45 mM potassium peroxydisulfate via incubation at room temperature in the dark for 12-16 h. The ABTS^+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.060 at 734 nm. To 3 mL of diluted ABTS^+ , 30 μL of sample solution were added and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was recorded immediately at 734 nm. Trolox standard solutions in corresponding solvent were prepared and assayed using the same conditions. Appropriate solvent blanks were run in each assay. The absorbance of the resulting oxidized solution was compared to that of the calibrated trolox (TE) standard. The free radical-scavenging activity of each extract was measured in terms of trolox equivalent antioxidant capacity (TEAC) and expressed as $\mu\text{mol TE per g}$ of dry or fresh matter (Re *et al.*, 1999).

2.7. Statistical analyses

Different statistical techniques such as analysis of variance (ANOVA), Duncan's multiple range method and Pearson's correlation were carried out for analyzing the data obtained from different types of solvent extraction and to study the relationships between different analytical parameters. The Duncan test was used to compare the effect of the solvent type on total phenols and flavonoids (Table 1) and on the trolox equivalent antioxidant capacity (Figure

2). The Principal Component Analysis (PCA) was performed to evaluate the efficiency of solvent on the extraction of phenolics and antioxidant activities. The statistical analysis was performed using the XLSTAT software, Version 2009.4.03 (Addinsoft) and the SPSS 13.0 for windows (SPSS Inc., 2004). Results are shown as the mean values and standard deviations of independent measurements ($n = 6$) and a difference was considered statistically significant when the p value was < 0.05 .

3. RESULTS AND DISCUSSION

3.1. Total phenol and flavonoid contents

The total phenol (TP) and flavonoid (TF) contents of olive leaves, obtained using various solvents: 80% MeOH, 70% EtOH, 80% acetone and ddH₂O are shown in Table 1. The means of TP in olive leaf extracts in terms of mg GAE g^{-1} FM and DM were ranged from 9.07 to 13.68 mg and 16.52 to 24.93 mg, respectively. These results are comparable to those reported by Boudhrioua *et al.* (2009) for Chétoui olive leaves (2.32 ± 0.11 g C.A. 100 g^{-1} DM, i.e. 23.2 ± 0.11 mg C.A. g^{-1} DM). Our results showed that methanolic, ethanolic and acetone extracts had the highest amount of phenolic content while ddH₂O extract contained the lowest amount.

The TF content, expressed as mg CE g^{-1} FM and DM, varied from 3.42 to 11.78 mg and 6.23 to 21.47 mg, respectively. It significantly ($p < 0.05$) depends on the solvent used as exhibited by Duncan's multiple range test (Table 1). Our data revealed that 80% MeOH is the most effective solvent for flavonoid extraction from olive leaves. According to these results TF content followed this order: 80% MeOH > 70% EtOH > 80% acetone > ddH₂O. Our data are in accordance with those reported in the literature which showed that methanol had better recoveries (Lihu *et al.*, 2004) and is specifically effective in extracting polyphenols (Pinelo *et al.*, 2004). Similarly, Manian *et al.* (2008) found that the methanol extract of green tea and methanol extract of *Ficus racemosa* (stem bark) contained relatively higher levels of total phenolics than the other extracts. Furthermore, MeOH 70-80% has

Table 1
Total phenols and flavonoids in olive leaf extracts expressed as mg of gallic acid (GAE) and catechin (CE) per g of fresh (FM) or dry matter (DM)

| | Total phenols | | Total flavonoids | |
|--------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | mg GAE g^{-1} FM | mg GAE g^{-1} DM | mg CE g^{-1} FM | mg CE g^{-1} DM |
| 80% MeOH | 13.23 \pm 0.40 ^b | 24.09 \pm 0.73 ^b | 11.78 \pm 1.41 ^d | 21.47 \pm 2.56 ^d |
| 70% EtOH | 13.37 \pm 0.47 ^b | 24.36 \pm 0.85 ^b | 8.69 \pm 0.69 ^c | 15.83 \pm 1.26 ^c |
| 80% acetone | 13.68 \pm 0.42 ^b | 24.93 \pm 0.77 ^b | 7.32 \pm 0.65 ^b | 13.33 \pm 1.19 ^b |
| ddH ₂ O | 9.07 \pm 0.34 ^a | 16.52 \pm 0.62 ^a | 3.42 \pm 0.34 ^a | 6.23 \pm 0.62 ^a |

^a Results are means \pm SD of six replicates. ^b Values that are followed by different letters in the same row are significantly different ($p < 0.05$).

produced good yield in extracting hydroxycinnamic derivatives, flavones, flavonols and catechins from fruits, legumes, grape seeds and wine pomace (Spigno *et al.*, 2007). Concerning the efficiency of the other solvents used, contradictory results are reported in the literature. A study conducted on olive leaves by Altiok *et al.* (2008) revealed that the TF content in 70% aqueous ethanol was slightly higher than the total phenol content in a 90% aqueous acetone solution. Moreover, Rusak *et al.* (2008) reported that 40% ethanol is the most effective among the solvents tested in the prolonged extraction of catechins, especially in the extraction of EGCG, the dominant catechin in tea leaves. Nevertheless, other researchers revealed that acetone and aqueous acetone were the best solvents for the extraction of polyphenols, flavonoids and condensed tannins (Drużyńska *et al.*, 2007; Liu *et al.*, 2009). On the other hand, Ho, *et al.* (2008) reported that water and ethanol extracts of longan flowers having high levels of total phenols, total flavonoids and condensed tannins exhibited better antioxidant ability than its water extract. Also, a study on pummel and navel oranges showed that the highest phenolic content was obtained from ethyl acetate and the minimum phenolic content was found in methanol extract (Jayaprakasha *et al.*, 2008). Thus, as it is deduced by some authors (Naczek and Shahidi, 2006; Zarena and Sankar, 2009), the recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility. The least polar solvents are considered to be suitable for the extraction of lipophilic phenols unless very high pressure is used (Allothman *et al.*, 2009).

3.2. Antioxidant activities

Since the methods used to measure antioxidant activity are extremely dependent on the reaction conditions and the substrates or products, not all methods yield the same values for the activity (Fukumoto and Mazza, 2000; Ou *et al.*, 2002). In order to obtain the most relevant data about the antioxidant capacity of olive leaf extracts, two different methods were used in this study.

Radical scavenging potential from OLEs at different concentrations was first tested by the DPPH method and the results are illustrated in Figure 1. All extracts are a source of radical scavenging activity (RSA), this activity changed with the solvent used to prepare the extract. Our results showed that the RSA increased with the extract concentration. The 80% MeOH extract exhibited significantly higher RSA. It seems that the results are clearly different even at a low concentration. For example, at 0.5 mg mL⁻¹, the RSAs were 93.70%, 59.74%, 40.20% and 20.83% for 80% MeOH, 70% EtOH, 80% acetone and ddH₂O extracts, respectively. Similar results are found by Sepúlveda-Jimenez *et al.* (2009), who found that for a same plant organ, extracts prepared with methanol possess a higher antiradical activity than those obtained with water. The IC₅₀ values of the DPPH radical-scavenging activity of OLEs expressed as mg of sample per mL were determined (data not shown). The lowest IC₅₀ value was for 80% MeOH (IC₅₀ = 0.17 mg mL⁻¹), which is also the richest in total flavonoids whereas the ddH₂O extract exhibited the weakest activity (IC₅₀ = 0.97 mg mL⁻¹). A lower IC₅₀ value indicates a high antioxidant activity and vice versa.

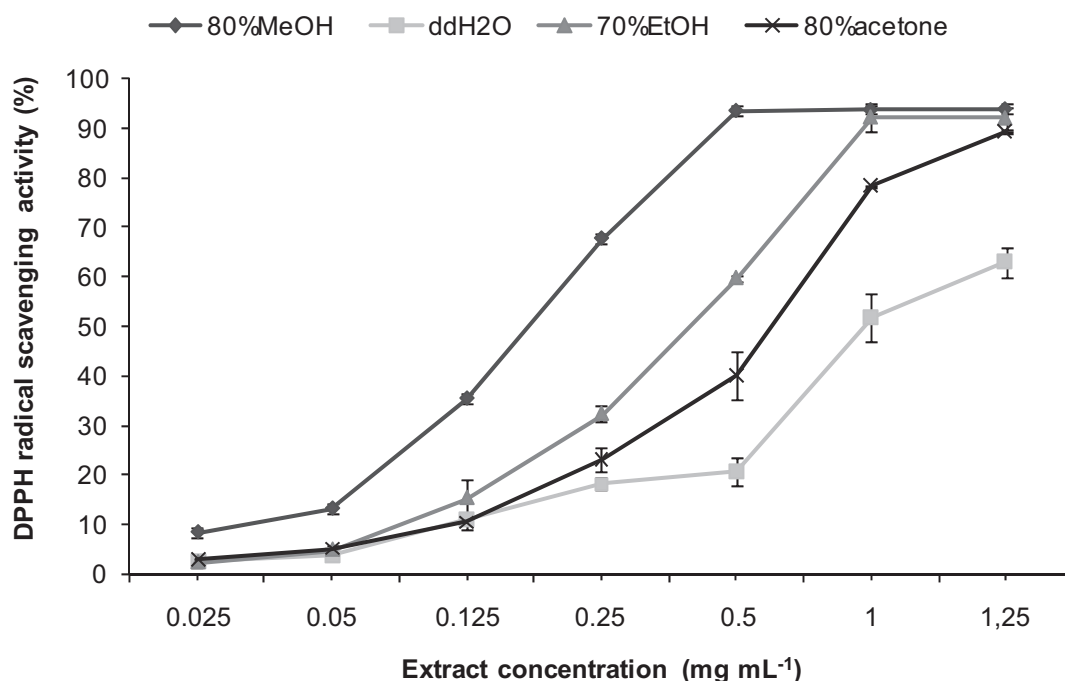


Figure 1
DPPH radical-scavenging activity of 80% MeOH, 70% EtOH, 80% acetone and ddH₂O olive leaf extracts. Each value is expressed as mean ± SD (n = 6)

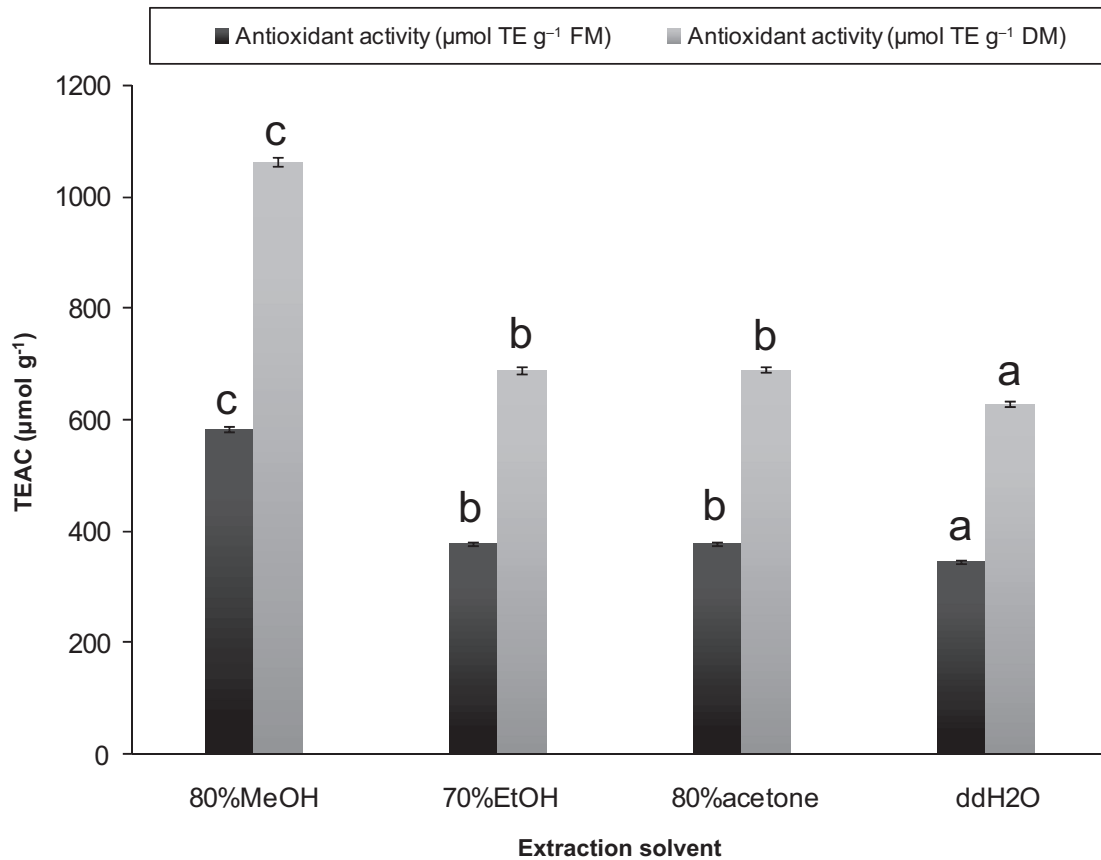


Figure 2
Trolox equivalent antioxidant capacity (TEAC) of 80% MeOH, 70% EtOH, 80% acetone and ddH₂O olive leaf extracts determined by ABTS. Each value is expressed as mean ± SD (n = 6). Antioxidant activity values followed by different letters are significantly different at p<0.05

The TEAC values determined by the ABTS of OLE from different solvents are shown in Figure 2. Three groups can be distinguished: (i) methanolic extracts with high TEAC values (584.27 μmol TE g⁻¹ FM corresponding to 1064.25 μmol TE g⁻¹ DM), (ii) ethanolic and acetone extracts with intermediate TEAC values and (iii) ddH₂O extracts with low TEAC values of 345.80 μmol TE g⁻¹ FM (629.87 μmol TE g⁻¹ DM). Olive leaf extract showed good levels of antioxidant activity when compared with *Ginkgo biloba*,

a well known phytochemical source of antioxidant, which displayed values of 312 and 274 μmol TE g⁻¹ dry weight for aqueous and methanolic extracts, respectively (Tawaha *et al.*, 2007). On the other hand, Thaiponga *et al.* (2006), studied the guava which is a fruit that has an exceptionally high antioxidant activity and reported that it did not exceed 40 μM TE g⁻¹ FM when extracted by methanol. Furthermore, the antioxidant activity of the methanolic extracts of some Jordan plant species ranged from 12.9 to 731 μmol

Table 2
Pearson's correlation coefficients (r) among the analysis parameters

| | TP mg GAE g ⁻¹ FM | TP mg GAE g ⁻¹ DM | TF mg CE g ⁻¹ FM | TF mg CE g ⁻¹ DM | IC 50 | TEAC (μmol TE g ⁻¹ FM) | TEAC (μmol TE g ⁻¹ DM) |
|-----------------------------------|------------------------------|------------------------------|-----------------------------|-----------------------------|------------|-----------------------------------|-----------------------------------|
| TP mg GAE g ⁻¹ FM | | 1.000(**) | 0.766(**) | 0.766(**) | -0.778(**) | 0.399(*) | 0.399(*) |
| TP mg GAE g ⁻¹ DM | | | 0.766(**) | 0.766(**) | -0.778(**) | 0.399(*) | 0.399(*) |
| TF mg CE g ⁻¹ FM | | | | 1.000(**) | -0.958(**) | 0.821(**) | 0.821(**) |
| TF mg CE g ⁻¹ DM | | | | | -0.958(**) | 0.821(**) | 0.821(**) |
| IC50 | | | | | | -0.794(**) | -0.794(**) |
| TEAC (μmol TE g ⁻¹ FM) | | | | | | | 1.000(**) |
| TEAC (μmol TE g ⁻¹ DM) | | | | | | | |

^a ** Correlation is significant at the 0.01 level (p<0.01). ^b *Correlation is significant at the 0.05 level (p<0.05).

TE g⁻¹ dry weight (Tawaha *et al.*, 2007). Our data are much higher than those reported for other fruits. Wang *et al.* (1996) reported the antioxidant activity of 12 fresh fruits (melon, pear, tomato, apple, banana, white and pink grape, pink grapefruit, orange, kiwi, plum, strawberry) ranging from less than 1 mM TE g⁻¹ FM for melon up to 15 mM TE g⁻¹ FM for strawberry.

3.3. Correlations between antioxidant properties and total phenols and flavonoids

Correlations were tested to link the antioxidant activities measured by the DPPH and ABTS methods as well as with the phenol and flavonoid contents of OLE. Table 2 summarizes the Pearson's correlation coefficients between all analyses carried out on the extracts. Our study showed a good correlation between TP and TF expressed as DM and FM ($r = 0.766$). Also we noticed a significantly negative correlation between IC50 values, TP ($r = -0.778$) and TF ($r = -0.958$). In previous works, authors (Velioglu *et al.*, 1998; Barros *et al.*, 2007; Barreira *et al.*, 2008) observed a significantly negative linear correlation between the polyphenol contents and EC50 (effective concentration) values confirming that phenolics are likely to contribute to the antioxidant activity of the extracts. However, they found that there is a poor or no correlation between the flavonoid contents and EC50 scavenging capacity. Recently, Kalyoncu *et al.* (2009) observed a negative and good correlation between IC50 values and the total phenolic contents of apricots ($R^2 = 0.777$) ($p < 0.01$).

The TEAC values determined by the ABTS assay are consistent with the DPPH test represented by IC50 data ($r = -0.794$). Hence, both methods could be equally useful for assessing the antioxidant activities of OLEs. But although they are correlated, these two methods do not give exactly the same results: the TEAC of 70% EtOH and 80% acetone were practically the same while the IC50 value of the ethanol extract is less than the acetone one. Therefore, as it is mentioned by Tachakittirungrod *et al.*, (2007) the antioxidant components, existing may possess different predominant mechanisms of action related to their natural and their synergistic effects.

We observed a good positive correlation between TEAC and TF ($r = 0.821$). Nevertheless, poor correlation was observed in the case of TEAC and TP ($r = 0.399$). Our data are similar to those found on leaf extracts of *S. verticillata* by Matkowski *et al.* (2008) showing that the TEAC seems to depend more on the flavonoid content. The relationship between the chemical structure of flavonoids and their antioxidant activities had been analyzed by Van Acker *et al.* (1996) and Arora *et al.* (1998). According to those authors a catechol or a pyrogallol type moiety substitution on the B ring appeared to be essential for the antioxidant activities of flavonoids. The presence of a hydroxyl substituent on the flavonoid skeleton boosts activity, whereas a methoxyl substitution suppresses the antioxidant activity. So, the study of the flavonoidic

fraction will be very useful for the determination of the antioxidant capacity of our samples.

3.4. Chemometrics

All collected data were submitted to the Principal Component Analysis (PCA), which enables us to identify the suitable solvent extraction for each component. PCA provides a graphic representation on inter-sample (scores-plot) and inter-variable (loading-plot) relationships and provides a way to reduce the complexity of the data.

The first (PC1) and the second (PC2) principal component were sufficient to display the structure of the data since they explained 97.39% of the total variance. By examining the scores-plot (Figure 3) in the area defined by F1 and F2, we distinguished 3 independent groups, based on their extraction solvent. Group I, located on the left side of the scores-plot, consisted of ddH2O. Group II, located in the symmetrical position of the scores-plot, and represented by 70% EtOH and 80% acetone. Group III, located on the right-side of the scores-plot, included 80% MeOH.

Since the extracts were well-described by the scores-plot, the loading plot (Figure 4) was analyzed in order to show which variables influenced group separation. Group I was characterized by samples having the highest IC50 value (lowest RSA). Group II was characterized by extracts with the highest amounts of TP. Group III showed samples with the highest amounts of TF and important TEAC values.

The statistical analyses can explain the suitability of solvent extraction for phenols and the antioxidant properties of olive leaf extracts. The best trolox equivalent antioxidant capacity and total flavonoid values were obtained using an 80% MeOH solvent while the lowest radical scavenging activity is obtained by water extraction.

4. CONCLUSIONS

This study demonstrated clearly that Chétoui olive leaves are a good source of phenols particularly flavonoids which possess potent antioxidant activity. Our results denoted that methanol is the most efficient extraction solvent. The high antioxidant activity of such an extract was attributed to the radical scavenging mechanism. Furthermore studies on the isolation and quantification of individual phenolic compounds are required to help research concerning the bioavailability of these flavonoids for epidemical and clinical studies.

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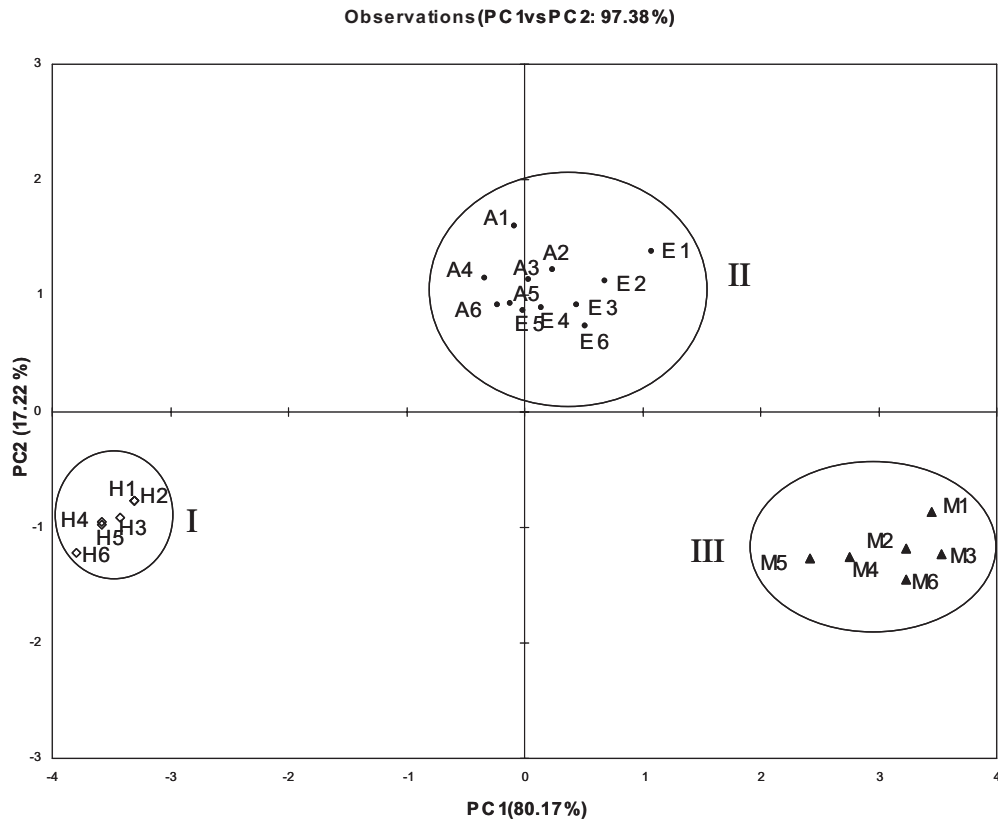


Figure 3
Scores-plot from a PCA applied to the extraction solvents showing Group I (◊), Group II (●) and Group III (▲). H: ddH₂O, A: 80% acetone, E: 70% EtOH and M: 80% MeOH

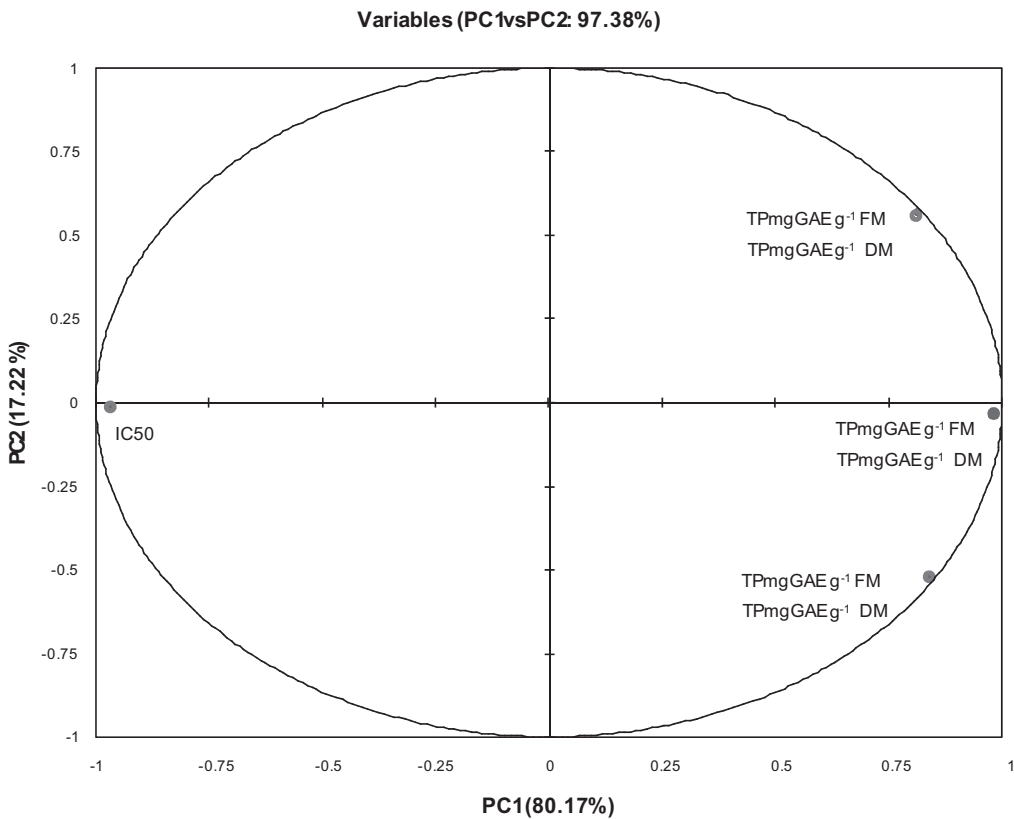


Figure 4
Loading-plot from a PCA applied to all the studied parameters

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