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# Antioxidant Capacity and Phenolic Content of Olive Fruits from Tunisia, Extracted with Different Solvents

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**Abstract:** The antioxidant capacity and phenol content of the olive fruits from the variety *chetoui*, were studied. Four solvent systems were used; hexane, dichloromethane, ethyl acetate and methanol. The antioxidant capacity of the fruit extracts was evaluated using 2, 2-diphenyl-1-picrylhydrazyl radical-scavenging assays. The efficiency of the solvents used to extract phenols from the fruits varied considerably. The results indicated that the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in the olive fruit.

**Keywords:** *Olea europaea* L., Anthocyanin, Caroenoids, Phytochemical Composition, Antioxidant Activity

## 1. Introduction

Nowadays, the consumption of fruit and vegetables is considered as important and beneficial for health. In fact, many epidemiological studies have demonstrated that a high intake of fruits and vegetables is inversely associated with morbidity and mortality from several chronic diseases such as cancer, cardiovascular diseases, coronary heart diseases, aging, atherosclerosis, neurodegenerative diseases (such as Parkinson and Alzheimer) and inflammation [1-6]. These protective effects have been paticularly attributed to various antioxidant compounds, such as vitamins C and E, ß -carotene, and polyphenolics [7]. Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases [8].

Olive oil plays an important role in the Tunisian agronomy and economy. Olive trees cover an area of 1,611,200 ha and account for more than 4% of the olive oil produced in the world. Indeed, Tunisia is the fourth largest exporter of olive oil in the world [9]. Due to the diversity of Tunisian olive oil cultivars, the olive-growing areas spread from north to south, where a wide range of pedoclimatic conditions prevail, from mild semi-arid to arid conditions [10]. More than 50 different cultivars are found throughout Tunisia; however, two important varieties dominate most of the arable land: *chetoui* and ch [9]. The Chetoui variety can be found mostly in the north of the country covering an area of 176,000 ha [11]. Its fruit is medium to large in size, with a characteristically elongated and asymmetric shape. The fat yield is 20–30% of fresh weight and the oil is valued for its high amounts of total phenols and tocopherols [11]. It has long been known that the chemical composition of olive is influenced by genetic (cultivar) and environmental factors (edaphic characteristics and climatological conditions), so that the olive production area is greatly responsible for the specific characteristics of olive oil. In the last few years, there has been increasing interest in the geographical identification of virgin olive oil, as a reliable criterion for its authentication and quality. Therefore, detailed information about the health promoting components of the variety *chetoui* could lead to a better understanding. The present investigation was carried out to evaluate the antioxidant activity of hexane, dichloromethane, ethyl acetate and methanol extracts of olive fruits. Additionally, total phenolic and flavonoid contents of hexane, dichloromethane, ethyl acetate and methanol extracts have been determined. An attempt was also made to relate the potential antioxidant activity with

the total phenolic content.

# 2. Materials and Methods

### *2.1. Plant and Sample Material*

Fresh fruits of the cultivar *chetoui* of *O. europaea* L. were collected from the north (Beja) of Tunisia. Olive fruits were isolated manually from the aerial parts in our laboratory to obtain a weight of 500–700 g of each part. Part of fruits has been dried in the shadow at room temperature (25 °C), for 8 days.Voucher specimens have been deposited in the Herbarium of the Laboratory of Biochemistry, Faculty of Medicine of Monastir, Tunisia.

### *2.2. Determination of the Anthocyanin Content*

Anthocyanin content of Olive fruits was analyzed according to Padmavati et al. (1997) [12] modified by Chung et al. (2005) [13]. The fruits (1 g) were extracted with 25 mg/ml of acidified methanol (1% HCl) for 2 h at room temperature in the dark, and then centrifuged at 1000g for 15 min. Anthocyanin levels were calculated from the methanolic extract as A530−(0.24 × A653) [14]. Total anthocyanin content was determined as mg cyanidin 3-glucoside equivalents (CyE) per 100 g of fresh weight , using an extinction coefficient of 26.900 l $mol^{-1}$ $cm^{-1}$ at 530 nm and a molar mass (MW) of 449.2 g $mol^{-1}$ [15].

$$\text{Anthocyanin (mg/100g)} = \frac{\text{Ab}\times\text{MW}\times\text{V}\times 100}{\varepsilon\times G}$$

### *2.3. Measurement of Total Carotenoids*

Total carotenoids were extracted according to the method of Talcott and Howard (1999) [16], with slight modifications. Two grams of sample fruit was extracted using 25 ml of acetone/ethanol (1:1, v/v) with 200 mg/l butylated hydroxytoluene (BHT) added. All manipulations were carried out under a yellow fluorescent light (Thorn), to avoid light-induced changes. After extraction, sample was centrifuged at 1500 × g for 15 min at 4–5°C. The supernatant was collected, and the remaining residue was re-extracted using the same method until the residue was colorless. Finally, the combined supernatants were brought to 100 ml with the extraction solvent, and the absorbance at 470 nm was measured using a UV-1601 Shimadzu spectrophotometer (Shimadzu). Total carotenoids were calculated according to the method of Gross (1991) [17], using the following equation, and expressed as mg/100 g of dry weight.

$$\text{Anthocyanin (mg/100g)} = \frac{\text{Ab} \times \text{V} \times 106}{\text{A1\%} \times 100\ \text{G}}$$

Ab is the absorbance at 470 nm, V is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a 1% mixture of carotenoids at 2500, and G is sample weight (g).

### *2.4. Preparation of the Extracts*

Extracts of air-dried plant materials were prepared by using solvents of varying polarity and the extraction protocol of each is given below. A portion (100 g) of dried plant material was extracted with hexane (HE), followed by dichloromethane (DCM), ethyl acetate and methanol in a Soxhlet apparatus (6 h for each solvent) [18]. In this procedure, starting material was first extracted with hexane, and after that, the same solid material with dichloromethane, ethyl acetate and methanol (subsequently). The fruits extracts were then transferred to vials and kept at _20 °C.

### *2.5. Total Phenols and O-diphenols*

Total phenolic contents and *o*-diphenols of fractions were determined according to the method of Montedoro et al. (1992) [19] with minor modifications. For total phenols, 0.4 milliliter of each fraction and 10 ml of diluted Folin–Ciocalteu reagent were mixed. After 1-min incubation, 8 ml of sodium carbonate (75g/L) was added and the mixture was incubated for 1 h. The absorbance was measured at 765 nm. The same extract was used to determine total *o*-diphenols. Then, 1 ml of a solution of HCl (0.5 N), 1 ml of a solution of a mixture of $NaNO_2$ (10 g) and $NaMoO_4.2H_2O$ (10 g) in 100 ml $H_2O$ and finally 1 ml of a solution of NaOH (1 N) were added to 100 µl of the leaves extract. After 30 min, *o*-diphenols were read at 500nm. The total phenols and o-diphenols were expressed on a dry weight basis as mg hydroxytyrosol equivalents / 100 g of sample.

### *2.6. Determination of Total Flavonoïds*

Total flavonoïd contents (TF) of the fruits extracts were determined according to the colorimetric assay developed by Zhishen et al. (1999) [20]. One ml of properly diluted fruits extract was mixed with 4 ml of distilled water. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH were added. Finally, the volume was made up to 10 ml, immediately by the addition of 2.4 ml of distilled water. The mixture was shaken vigorously and the absorbance was read at 510 nm. The results were also expressed on a dry weight basis as mg catechin equivalents (CEQ) / 100 g of sample.

### *2.7. Antioxidant Activity (DPPH Radical Scavenging Assay)*

The DPPH method [21] was used to determine antioxidant activity of olive fruits extracts. 20-µL from the stock solution of the sample were dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol). The reaction mixture was kept at roomtemperature. The optical density (OD) of the solution was measured at 517 nm, after 60 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction values were determined and compared to appropriate standards. Inhibition of the free radical DPPH, in percent (I %) was calculated using the following equation:

$$I\% = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

Where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the tested compound), and $A_{sample}$ is the absorbance of the tested compound.

### *2.8. Statistical Analyses*

All assays were run in triplicate. The results are reported as mean values of three analysis and standard deviation. Data were subjected to statistical analysis using the SPSS programme, release 11.0 for Windows (SPSS, Chicago, IL, USA). The one-way analysis of variance (ANOVA) followed by Duncan multiple range test were employed to study the effect of solvent and the differences between individual means were deemed to be significant at $p < 0.05$.

## 3. Results and Discussion

### *3.1. Anthocyanin and Carotenoid Contents*

Carotenoids are widely distributed among coloured fruits and vegetables and contribute to both the appearance and attractiveness of fruit as well as additional nutritional value in the form of dietary antioxidants [22]. The total amount of carotenoids exceeds 20 mg/100 g DW for *chetoui* cultivar. Therefore, data showed that *chetoui* had the higher level of carotenoids (23.4 mg/100 g DW), whilst lower levels of anthocyanin were detected in *chetoui* (0.06 mg/100 g DW). Epidemiological studies have shown that high intakes of carotenoid-rich vegetables and fruits and high blood levels of ß-carotene are associated with decreased incidence of some cancers [23].

### *3.2. Phytochemical Composition*

Total phenolic content of the fruit extracts were determined using a method based on Folin–Ciocalteu reagent reduction. Results, expressed as milligrams of hydroxytyrosol equivalents per 100 grams of dry weight, were 313.56, 309.77, 36.05 and 83.32 mg/100g DW for hexane, dichloromethane, ethyl acetate and methanol of the fruit extract, respectively (Fig. 1A). These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [24]. The phenolic compounds may contribute directly to antioxidative action [25]. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables [26].The amount of total phenolics was highest in the hexane extract, followed by the dichloromethane, methanol and ethyl acetate extracts. In fact, the lowest value was exhibited by the ethyl acetate extract. Phenolics were found to be one of the most plentiful classes of constituents in hexane extract of olive fruits from the cultivar *chetoui*. This is due to the presence of high bioactive compounds in hexane extract as compared to other organic extracts and due to the wide range of phenols that the hexane and dichloromethane can dissolve. In fact, the results given in Fig. 1 showed significant differences between the solvent extracts. These results are in agreement with report by [27], who showed that the least polar solvents are considered to be suitable for the extraction of lipophilic phenols unless very high pressure is used.

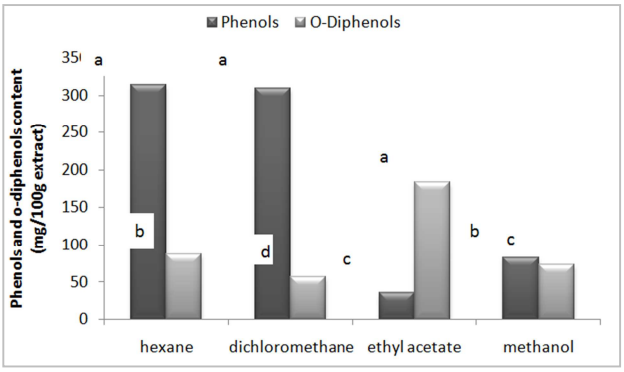


***Fig. 1.*** *Total phenolic content of extracts of olive fruits by different solvent extraction. Results are expressed as hydroxytyrosol equivalents. Values are expressed as means ± standard deviation (n = 3). Means with different letters were significantly different at the level of p < 0.05.*

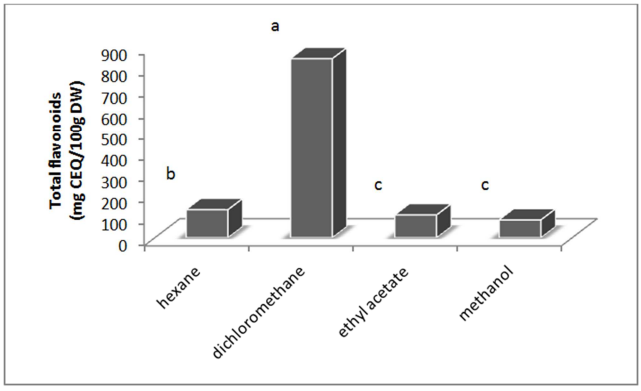


***Fig. 2.*** *Total flavonoids content (as catechin equivalents) in extracts from olive fruits. Values are expressed as means ± standard deviation (n = 3). Means with different letters were significantly different at the level of p < 0.05.*

Total o-diphenols of the fruits ranged from 57.01 to 183.37 mg/100 g DW for hexane, dichloromethane, ethyl acetate and methanol extracts, respectively (Fig. 1). Statistically, the results showed significant differences ($p \leq 0.05$), among solvents extracts. Therefore, ethyl acetate and hexane extracts had higher o-diphenols contents when compared with the other two extracts. Earlier, solvents, such as methanol, ethanol, acetone, propanol, ethyl acetate and dimethylformamide, have been commonly used for the extraction of phenolics from plants [28-29]. From the results shown in Fig. 1, it is evident that the recovery of o-diphenols content was dependent on the solvent used and its polarity. Moreover, hexane was found to be an efficient solvent for polyphenol and o-diphenols extraction. Therefore, it is hard to develop a standard extraction procedure suitable for the extraction of all plant phenols.

The total flavonoid (TF) content of the fruits from the cultivar *chetoui* was determined (Fig. 2). Dichloromethane and hexane recover the highest content of TF (844.62 and

131.17 mg CEQ/100 g DW, respectively) in olive fruits extracts with significant difference ($p \leq 0.05$) when compared with all other solvent systems used. The flavonoids are an important phenolic group in representing the antioxidant capacity [27]. The results in Fig. 2 showed that the total flavonoid was dependent on the solvent used. Furthermore, dichloromethane have acceptability for human consumption models. On the other hand, hexane and dichloromethane are good solvent systems for the extraction of polar antioxidants.

### *3.3. Antioxidant Activity (DPPH Radical Scavenging Activity)*

There are a huge varieties of antioxidants contained in fruits. Therefore, measuring the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity of different plant materials [30]. With regard to the olive fruits extracts, Fig. 3 illustrates that the sequence for DPPH radical-scavenging ability was dichloromethane extract > ethyl acetate extract > methanol extract> hexane extract. The results showed that the % inhibition for the dichloromethane and ethyl acetate extracts were 72.3 and 68.74%, respectively with no significant differences ($p > 0.05$) in antioxidant activities were found between them, indicating that extracts with different solvents displayed a similarly strong antioxidant activity. This is due to most bioactive compounds such as polyphenols and flavonoid existed in polar extracts [31]. Polyphenols are one of the major plant compounds with antioxidant activity. Therefore, this order is similar to the phenolic contents of the extracts that showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in the extracts. In fact, organic extracts may be more beneficial than isolated constituents, because other compounds present in the extracts can change the chemical or biological properties of bioactive individual component [32].

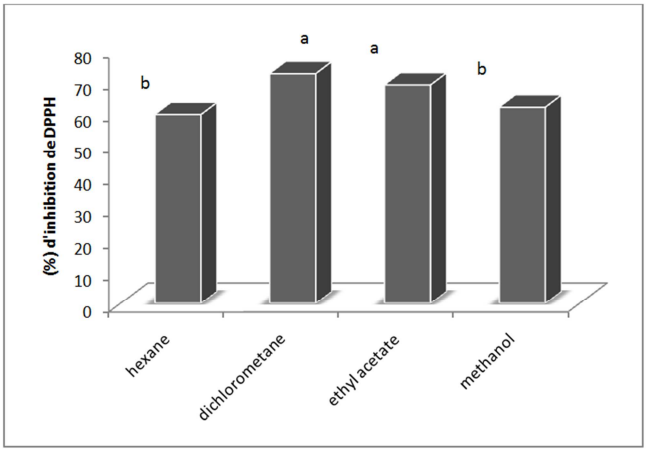


***Fig. 3.*** *Free radical-scavenging capacities of the extracts measured in DPPH assay. Values are expressed as means ± standard deviation (n = 3). Means with different letters were significantly different at the level of $p < 0.05$.*

## 4. Conclusion

The recovery of phenols from the olive fruit was dependent on the solvent system used. Hexane and ethyl acetate were the most efficient solvents for extracting *o*-diphenols from olive fruits, while both hexane and dichloromethane efficiently extracted polyphenols and flavonoids from the fruits. Dichloromethane and ethyl acetate extracts showed the highest scavenging activities against DPPH. The data on extraction procedures and antioxidant activity assessment obtained in these experiments single out ethyl acetate and dichloromethane as the most promising sources for the isolation of natural antioxidative compounds from the olive fruits.

## Acknowledgments

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