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Antioxidant Potential of olive *(Olea europaea* L.) from Algeria

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ABSTRACT

olive fruit (Olea europaea L.) from the Algeria were powdered and extracted why to assess, the one hand, total phenolic and flavonoid content and, on the other hand, antioxidant activity. The results showed higher levels of total phenolic (19. 97± 0.76 mg PE/g dw in aqueous methanol and 15.81± 0.16 mg PE/g dw in aqueous acetone). Higher Free Radical Scavenging and iron reducing power of ethyl acetate extract was observed (IC50, 0.0478 ± 0.003mg/ml and 0.206 ± 0.026 mg/ml respectively).

Keywords: *Olea europaea* L, phenolic, flavonoid, antioxidant activity.

INTRODUCTION

Olive tree (*Olea europaea L.*) is one of the most important fruit trees in Mediterranean countries, where they cover 8 million ha, accounting for almost 98% of the world crop [1].This demonstrates the great economic and social importance of this crop and the possible benefits to be derived from utilisation of any of its byproducts [2, 3].

The phenolics content of olive depends on several factors, such as cultivar [4, 5], climate [6], irrigation regimes [7], degree of ripeness of the fruit [8], and elaboration process [9]. Recently there is an increasing interest in olive products and byproducts, due to their antioxidant properties. Many studies describe phenolic compounds as having a protective role in the oxidation of low-density lipoproteins [10] and in oxidative alterations due to free radical and other reactive species [11].

In Algeria, there is no study on olive table which grows on a large area. Herein, we intended to evaluate the phenolic compounds of Sigoise variety table olives produced in Algeria. We also intended to correlate the phenolics levels with the antioxidant activity of the table olive extracts.

The antioxidant activity was evaluated by two chemical assays: reducing power and scavenging effects on DPPH radicals.

MATERIALS AND METHODS

Plant extraction

5 g, 5g and 10g of defatted powder of the olives was weighted into adequate glass beaker and 100 ml of Extract of aqueous acetone (70%), 100 ml of Extract of aqueous methanol (80%) and 200 ml of Extract aqueous methanol (80%) were added. The beakers were suspended in a water bath and homogenized with an (ULTRATURRAX, IKAR WERKE) at 13500 rpm for 30 min at 4°C. The content of each beaker was filtered separately through filter paper. The residue was again treated with similar manner.

Extraction of flavonoids

Sample extracts of aqueous methanol were evaporated to dry under reduced pressure at 45°C. The dried weight obtained were measured and treated with 10 ml of hot distilled water in order to dissolve flavonoids. Then, they were extracted with ethyl acetate (3x10ml).The remaining extract was continuously extracted with n butanol (3x10 ml). Ethyl acetate extracts and n butanol extracts were washed with dried Na_2SO_4, and evaporated to dryness under reduced pressure at 45°c. The dried weight of each extract were measured and stored at 4°c for further tests [12].

Determination of total phenolic and flavonoid content

They were determined using extract sample of aqueous acetone and aqueous methanol.

Determination of total phenolic content

The amount of total phenolic content was determined by Folin-Ciocalteu procedure [13] .Aliquot (0.1 ml) of each sample extract was transferred into the test tubes and their volumes made up to 3 ml with distilled water. After addition of 0.5 ml Folin-Ciocalteu reagent and 2 ml of 20% aqueous sodium carbonate, tubes were vortexed and incubated at room temperature under dark condition. The absorbance was recorded after 1h at 650 nm JEN WAY 6405 UV/Vis spectrophotometer. The total phenolic content was calculated as a Pyrocatechol equivalent (mg PE/g DW), from the calibration curve of Pyrocatechol standard solutions (range 1-15 mg/ml), giving an equation as

Absorbance = 0.0132 Pyrocatechol (mg/ml) - 0.035 (R^2= 0.997)

All tests were carried out in triplicate.

Determination of total flavonoid content

It was determined based on the formation of flavonoid-aluminium [14] .1 ml of each sample extract was mixed with 1 ml 2% aluminium chloride solution. After incubation for 15 min at room temperature, the absorbance at 430 nm was determined in JEN WAY 6405 UV/Vis spectrophotometer. The calibration curve was performed with Rutine (range 0.1.1 mg/ml), giving an equation as

Absorbance = 2.302 Rutine (mg/ml) + 0,021 (R^2=0.992)

The results are expressed as Rutine equivalent (mg QE/g DW). Tests were carried out in triplicate.

Antioxidant activity

The antioxidant activity of ethyl acetate fraction, butanolic fraction, aqueous acetone extract and aqueous methanol extract of olive were assessed.

(1) Free radical scavenging activity

The procedure of [15] was adapted for evaluation of the free-radical scavenging capacity of sample extracts. Briefly, dried extracts were dissolved in methanol why obtained different concentration (to 0.01mg/ml until 1mg/ml) of each one. The assay mixture contained in total volume of 1 ml, 500 µ1 of the extract, 125 µl prepared DPPH (1mM in methanol) and 375 µl of methanol. Ascorbic acid was used as a positive control. After 30 min incubation at 25°C, the decrease in absorbance was measured at λ =517 nm in JEN WAY 6405 UV/Vis spectrophotometer against blank of each concentration (extract plus methanol).The capacity to scavenge the DPPH radical was calculated as follows:

Where, A is the absorbance of the negative control (DPPH plus methanol) and B is the absorbance of the sample (DPPH, methanol plus sample). The correlation between each concentration and its percentage of scavenging was plotted, and the EC50 was calculated by interpolation. The activity was expressed as EC50 (the effective concentration of each extract that scavenges50% of DPPH radicals).

(2)Iron reducing power

The capacity of plant extracts to reduce Fe3+ was assessed by the method of [16]. Each dried extract were dissolved with methanol and different concentration (0.1, 0.25, 0.5, 0.75 and1mg/ml) were prepared. One milliliter of each one was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 3000g for 10 min. The upper layer fraction (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of0.1% ferric chloride and thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. The EC50 value (mg/ml) is the extract concentration at which the absorbance was 0.5 for the reducing power and was obtained from the linear regression equation prepared from the concentrations of the extracts and the absorbance values. A higher absorbance indicates a higher reducing power.

Statistical analysis

Assays were performed in triplicate for each sample. Results were expressed as mean values± standard deviation (SD). Correlation and regression analysis were carried out using the Origin Pro 8 SRO v8.0724 (B724).

RESULTS AND DISCUSSION

Table 1- Field of different extracts from olives fruits

Extract	Olives fruits %
Ethyl acetate extract	3.00 ± 0.05
Butanolic extract	9.1 ± 0.01
Methanol extract	24.65 ± 0.01
Acetone extract	29.00± 0.06

Extract yield

Table 1 showed the extraction yielding obtained for each extraction from olives. We observed that the highest yield is of Acetone extract (29. 00± 0.06%) compared with Methanol extract (24. 65 ± 0.01%), Butanolic extract (9.1 ± 0.01%) and Ethyl acetate extract (3.00 ± 0.05%).

Determination of total phenolic and flavonoid content

From Table 2, there were differences in total phenolic and flavonoid contents of different extracts of olives, depending on solvents. The highest levels of total phenolic and flavonoid contents were found in aqueous methanol (with 19. 97± 0.76 mg PE/g dw for phenolics and 0.2865 ± 0.0170 mg RE/g dw for flavonoids) while total phenolic and flavonoid contents of aqueous acetone were lowest, 15.81± 0.16 mg PE/g dw and 0.1045 ± 0.003 mg RE/g dw, respectively.

Table 2- Total phenolic and flavonoid of different extract from olives fruits

Extract	Total phenols(mg PE/g dw)	Total flavonoids (mg RE/g dw)
Aqueous acetone	15.81± 0.16	0.1045 ± 0.003
Aqueous methanol	19. 97± 0.76	0.2865 ± 0.0170

Antioxidant activity

(1) Free radical Scavenging activity

The results of DPPH (TABLE 3) test showed that Ethyl acetate extract was the most active with an IC50 value of 0.0478 ± 0.003 mg/ml followed by Methanol extract, Acetone extract and Butanolic extract with IC50 values 0.1484 ± 0.009, 0.1508 ± 0.011and 0.2707 ± 0.012 mg/ml, respectively. These plant extracts showed lower radical scavenging activity compared to Ascorbic acid (IC50, 0.0106 ± 0.002 mg /ml).

(2) Iron reducing power

Iron reducing power of a compound may serve as indicator of its potential antioxidant activity. As shown in TABLE 3 the reducing power of Ethyl acetate extract , expressed as IC50, was higher than other extract plant (0.206 ± 0.026 mg /ml), followed by Acetone extract, Methanol extract and Butanolic extract with IC50 values of 0.2703 ± 0.015, 0.3054 ± 0.029 and 0.556 ± 0.033 mg/ml, respectively. Ascorbic acid was a higher reducing activity (IC50, 0.038 ± 0.0008 mg/ml).

Table 3 - EC Values (mg/mL) of olives extracts.

	DPPH (EC50)	**reducing Power (EC50)**
Ethyl acetate extract	0.0478 ± 0.003	0.206 ± 0.026
Butanolic extract	0.2707 ± 0.012	0.556 ± 0.033
Methanol extract	0.1484 ± 0.009	0.3054 ± 0.029
Acetone extract	0.1508 ± 0.011	0.2703 ± 0.015
Ascorbic acid	0.0106 ± 0.002	0.038 ± 0.0008

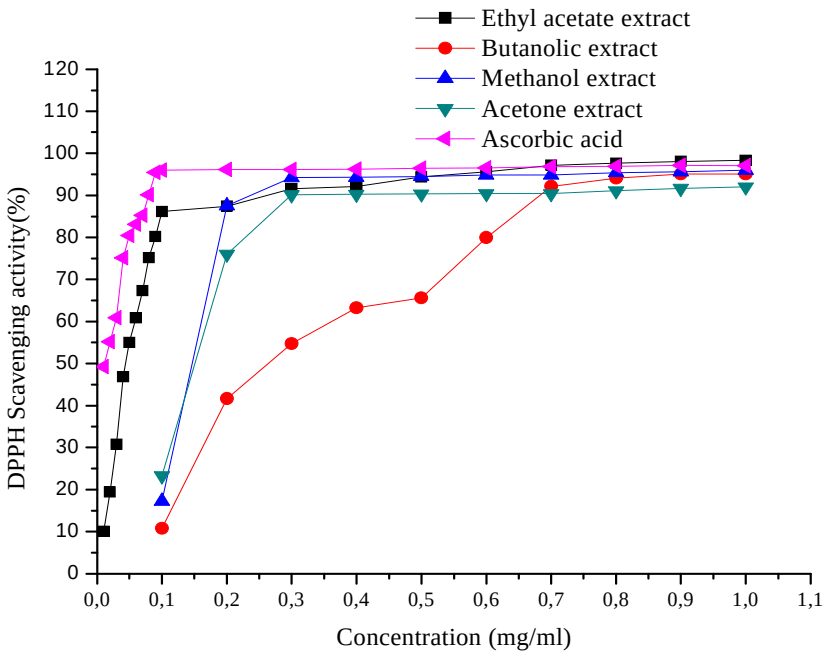

Figure 1- DPPH radical scavenging activities (%) of olives extracts. Ascorbic acid was used as positive control.

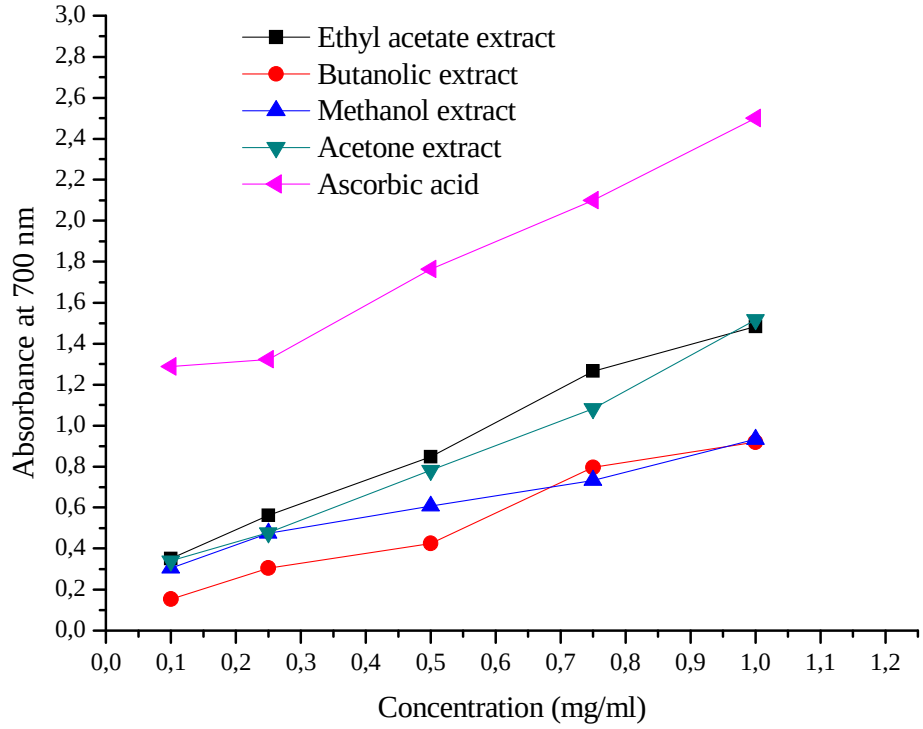

Figure 2 - Correlation between the sample concentrations and absorbance of reducing power of olives extracts. Ascorbic acid was used as positive control.

In this study, extract yield, total phenolic and flavonoid content and antioxidant activity of olives were determined.

Results of extract yield indicated that olive was a higher yield of flavonoids. In our study, by (ULTRA TURRAX, IKAR WERKE) extraction method, we have accelerated the process of extracting and minimized the time of contact with the extract solvent, while preserving the bio-activity of its constituents. Similarly, cold this temperature, extraction flow along the exhaustion of the solvent at reduced pressure, allows obtaining the maximum of compounds and preventing their denaturing, or likely change due to the temperatures used in other methods of extraction.

Phenolic compounds are found in all parts of the olive plant, but that nature and concentration varies greatly between the various tissues [17].The results revealed that the methanol extract contains significant more phenolic and flavonoids than of acetone extracts . [18] Founds that there is a relationship between the phenolic contents of different extract and DPPH radical-scavenging capacity, whereas a higher correlation between the total flavonoids contents and DPPH radical- scavenging capacity. In this study performed with olives, it is thought that the high free radical-scavenging activity and total antioxidant activity may result from the coexistence of phenolic and flavonoid-type compounds.

The levels of total polyphenol were superior to those reported by [4] (0.9 mg /g). The difference is probably due to olive varieties, climates and the degree of ripeness of fruit [4]. The phenolic composition of fruits is closely related to the variety [19, 20].

According to [4], the difference of total flavonoid, due to olive varieties, the degree of ripeness of fruit and the method of extracts. Flavonoids are a widely distributed group of polyphenolic compounds, identified in recent years as antioxidants in various biological systems [21].They are known to be synthesized by plants as a response to microbial attacks. They have been found in vitro to be effective against a wide range of microorganisms [22].

It had been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [23]. Our study demonstrated the higher activity of olive Ethyl acetate extract (Table 3). The strong antioxidant property of this extracts is associated to their phenols including falvonoids. The scavenging activity of flavonoids depends to a high degree on their structures and physicochemical properties [24]. [25] Reported that there is a correlation between phenolic contents of olive samples and the antioxidant activity.
Hydroxytyrosol, most probably, contributes to an important extent to the observed effects, as one of the phenolic compounds with higher antioxidant activity [26]. Nevertheless, other compounds, such as α-tocopherol, which is abundant in olive products and presents a high antioxidant activity, may be involved [27].

CONCLUSION

Olive is a potent source of polyphenols having antioxidant property. Olives may constitute a good source of healthy compounds, especially phenolics, in the diet, suggesting that their consumption could be useful in the prevention of diseases in which free radicals are implicated.

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