Phytochemical Screening and Antioxidant Activity of Leaf Extracts from Ten Algerian *Olea Europaea* L. Varieties

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Abstract
The aim of the current study is to investigate the content of some antioxidants extracted from ten olive leaf cultivars (*Olea europaea L*) collected from east of Algeria followed by the assessment of antioxidant activities of these extracts using TAC, DPPH and reducing power assays. The findings show that ethanolic extracts of all cultivars are rich in total phenolic and flavonoid compounds with maximums of (322.90 ± 11.04 and 122.00 ± 8.40 mg GAE/g DE), respectively. The *Aaleh* cultivar's ethanolic extract exhibits the highest levels of total antioxidant capacity (349.80 ± 14.00 mg AAE/g DW), the highest potent antioxidant power (A0.5 value of 49.96 ±0.27 µg/ml), and the most significant DPPH radical scavenging activity (IC50: 55.46 ± 2.56 µg/ml). In addition, the genotype of olive tree has a very high significant influence on the antioxidant amounts and activities. The results of this study provide a scientific basis for the traditional use of this local plants and demonstrate their potential as source of natural antioxidant bioactive compounds.

Keywords: DPPH, reducing power, phenolic, flavonoid.

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1. Introduction

Free radicals are extremely reactive molecules containing one or more unpaired electrons in their external orbitals. They are formed in different ways including enzymatic and non-enzymatic reactions which are related to protein oxidation and lipid peroxidation [1]. Thus, their excessive amounts in the body cause several disorders in humans such as: immune diseases, DNA mutation, central nervous system injury, cardiovascular diseases, diabetes, infection diseases, cancer[2]. Antioxidants (such as: carotenoids, flavonoids, and anthocyanidins) which are a set of molecules synthetized by plants to protect them from various environmental conditions [3], have the ability to reduce the negative effects of these reactive oxygen species that cause oxidative stress. Additionally, sufficient intake of antioxidants molecules is supposed to protect from human diseases that make them the focus of attention and interest in the therapeutic potentials of medicinal plants[4]. They

have the capacity to employ their act by several methodologies: scavenging free radicals, inhibiting their formation and preventing formation of hydroxyl and/or decomposition of lipid hydroperoxides and repairing damage.
In this context, olive leaves are one of the antioxidants stores mainly phenolic and flavonoid compounds. All these compounds have a protective function for the olive tree and for other plants in which they are formed[5].
Olive trees (*Olea europaea L.*) are largely cultivated in Algeria and their leaves have always occupied a significant position in the folk remedies in traditional medicine as a diuretic/the leaves are consumed mainly as a tea. Olive leaves have been reported beneficial health effects such as anti-hypertensive[6], hipoglycemiant[7], hypocholesterolemic[8], cardioprotective[9], anti-inflamatory[10].
Du to ethnobotanical significance of *Olea europaea* L. and the limited research on the leaves of our local cultivars that prove their potential antioxidant properties, this study was conducted to show the total antioxidant contents and to assess the antioxidant activities of the leaves extracts from ten Algerian olive cultivars.

2. Materials And Methods

1. Plant material

The olive leaves used as plant material are manually picked from ten olive cultivars *Olea europaea* L. (Aaleh, Aghechren d'ellousseur, Aghechren de Titeste, Agraraz, Blanquette de Guelma, Boughanfous, Neb Djmel, Tabelout, Takasrit, Tefah). Leaves are collected from experimental station of Technical Institute of Fruit and Vine Arboriculture (ITAFV) in TAKRIT, city of Bejaïa (Algeria) in March during the crop season 2021/2022 from mature trees. Plant identification was carried out by botanists at the institute of ITAFv. Samples were taken within the framework of an agreement between the university of El-Bachir El-Ibrahimi -Bordj Bou Arreridj-, and the institute of ITAFv in Algeria[11]. Three replicates from each collected samples are cleaned and dried at 25 °C in a shade for 15 days, powdered in electric grinder, then sieved to obtain a fine powder (<200 µ) that is stored afterwards in a dry place in the dark for extraction until analysis.

2. Preparation of olive leaf extracts

Using a magnetic stirrer and 150 ml of 70% ethanol, 10g of powdered olive leaves are extracted for 3 h at room temperature and in the dark. After completing the extraction process of different cultivars *Olea europaea* L. leaves, extracts are filtered through microfilters in order to obtain a clear crude extract solution. Again, the separation procedure is performed then the solvent is evaporated in a vacuum under 40 °C on a rotary evaporator. All the extraction processes are performed in triplicate. The extracts are frozen (-4 °C) for further analysis. The extraction yield (%) is determined as the ratio between the amount of dry extract obtained and the initial amount of powder used for extraction.

3. Physicochemical parameters

3.1. Extraction yield

The quantity of solid extract recovered in mass as opposed to the original amount of dry leaves at 40 °C in the oven is the extraction yield in (mg/g dry powder).

3.2. Determination of humidity Content

The moisture content of olive leaves is measured using AOAC (2000)[12] method. Firstly, one gram of the powdered olive leaves is taken into a crucible and weighed (m_1). It is then dispersed in a thin layer inside the moisture balancing device' crucible. Oven temperature is set to 105 °C. Until the moisture content of the plant material achieves a consistent weight (m_2), it is held at this temperature. The following formula is used to determine the percentage of humidity:

$H\% = (m_1-m_2/m_1) \times 100$.

3.3. Determination of ash content

After being dry mineralized in a porcelain crucible, 5 g of the powder from each plant is added to a furnace with ash at 550 °C, which is maintained at that temperature for 3 h. The ashes are taken out of the furnace, allowed to cool, and then preserved in a desiccator to maintain stable weights [13].

The ash content is calculated according to the following formula:

AC%= [M-M'/E] x 100

AC: ash content.

M: final mass (crucible+total ash).

M': mass of the empty crucible.

E: test sample of the material.

3.4. Determination of lipid content

The lipid content of our powdered material plant is determined by soxhlet extraction according to BIPEA[14]. Briefly, a round-bottomed flask is weighed (w_1) then filled with 2/3 petroleum ether. A quantity of 10 g from each powdered leaf is placed in an extraction cartridge (Wattman cartridge), which is afterwards inserted into the extraction bulb. Subsequently, the extraction is carried out by dissolving the fat with boiling petroleum ether (P.E. 35 °C). In the end, flask containing the fat is weighed again (w_2) after evaporating the ether on a rotary evaporator. The fat content is calculated according to the following formula:

$LC\ (\%) = [(w_2-w_1) \times 100] / SW$.

Whereas, LC: Fat Content. W_2: ball weight + mass of fat (g) dry. w_1: weight of empty balloon (g). SW: sample weight (g).

4. Phytochemical study

4.1. Determination of chlorophyll pigments content

The content of chlorophyll, lycopene, and β-carotene in leaf samples are determined using the method described by Nagata & Yamashita15 with modifications. 100 mg of each extract are mixed with 10 ml of the mixture acetone-hexane (4:6) and it is then vortexed to be homogenized uniformly. Subsequently, the whole mixture is filtered through Whatmann° 04 paper. After that, optical density of the supernatant at 663 nm, 645 nm, 505 nm and 453 nm are measured by spectrophotometer at the same time using Shimadzu™ UV-VIS 1800 Spectrophotemeter, USA. The amount of chlorophyll content is calculated in milligrams per gram of leaf tissues. From these values, the different pigments are estimated utilizing the following formula:

Chlorophyll 'a' (mg/100 mL) = 0.999 x A663 - 0.0989 x A645
Chlorophyll 'b' (mg/100 mL) = - 0.328 x A663+ 1.77 x A645

4.2. Determination of carotenoids pigments content

The method of Sass-Kiss et al.,[16] was slightly modified in order to determine the samples' total carotenoid content. To 5 g of dried plant material, 10 ml of a mixture of extraction solvents (hexane, acetone, and ethanol, 2:1:1) were added. The solution was first centrifuged for 30 min at 4500 rpm after 30 min of agitation. The supernatant liquid was then transferred into another vial, and the upper phase was recovered. A second extraction was performed with 10 ml of hexane. The total carotenoids were measured by spectrophotometry at 420 nm using the mixture of the two hexane phases. Using β-carotene as the standard, carotenoids concentrations are determined using the calibration curve, and the findings are given as µg/g dry weight (µg/g DE).

4.3. Determination of total phenolic compounds

The Folin Ciocalteu reagent method, as described by Singleton & Rossi [17], is used to determine the total phenolic content in the ethanol extracts of Olea Europaea L. leaves. 200 µl of sample or standard with appropriate dilutions is added to an aliquot of 1 ml of Folin's that has been diluted 10 times. 800 µl of a 7.5% sodium carbonate solution are added after 4 min. The combination is allowed to react for two hours at room temperature in the dark. The acquired data are assessed using the end acid calibration graph's linear regression equation. $Y= 0.0068 x + 0.056$. $R^2= 0.9991$. Where Y: Absorption Intensity. X: Total phenolic compounds expressed as gallic acid equivalent (mg GAE/g DE),

Shimadzu™ UV-VIS 1800 Spectrophotemeter, USA, a double beam UV-Vis spectrophotometer, is used to measure the absorbance of the solution and the blank at 760 nm. Three measurements are made again.

4.4. Determination of total flavonoids

The method developed by Jain et al.,[18] is used to estimate the total flavonols present in the leaf extracts. 1 ml of 2% $AlCl_3$ is added to 1 ml of each extract or standard solution at various concentrations. Using a double beam UV-vis spectrophotometer such as the Shimadzu™ UV-VIS 1800 Spectrophotemeter USA, the absorption at 430 nm is read after 1 h at room temperature. By adding 1 ml of quercetin at various concentrations, the classic quercetin diagram is created. Triplicate samples are used for each analysis. Using the following equation based on the calibration curve, total flavonoid levels are represented as milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE): $y = 0.333 + 0.024$; $R^2 = 0.9917$.
Where, y is the absorbance and x is the quercetin content ($\mu.ml^{-1}$).

4.5. Determination of condensed tannins

Concentrations of condensed tannin are determined by a modified version of a method reported previously by Oyedemi et al,.[19]. 1.5 ml of HCl (37% concentration) and 3 ml of vanillin solution (4%) in methanol are combined with 0.5 ml of each extract (1 mg/ml). At 500 nm, the absorbance in comparison to a blank is measured. Catechin is used to prepare the standard curve. The outcomes are given as mg of catechin equivalents per gram of dry extract.

5. Antioxidant activity

5.1. Total antioxidant capacity (TAC)

The method outlined by Prieto et al.,[20] is used to evaluate the total antioxidant capability of the extracts. A mixture of 3 ml of the reaction solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate) and an aliquot of 0.3 ml of each ethanolic extract (1 mg/ml) is added. The tubes are then incubated for 90 min at 95 °C. Shimadzu™ UV-VIS 1800 Spectrophotemeter, USA is used to test the absorbance at 695 nm against a blank solution after the combination has reached room temperature. The equation derived from a standard ascorbic acid calibration curve is used to calculate the total antioxidant capacity and represent it as mg ascorbic acid equivalents per gram of dry extract.

5.2. DPPH free radical scavenging activity

According to the procedure described by Burist & Bucar[21], the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay is used to assess the free radical scavenging activity of extracts. In a nutshell, 100 ml of methanol is used to dissolve DPPH to create a stock solution, which is then diluted to produce an absorbance of 0.98 ± 0.04 at 517 nm. The control without the test sample is infused with an identical volume (2 ml) of ethanol. To 2.5 ml of DPPH, 100 µl of each extract at various standards or concentrations (ascorbic acid and butylated hydroxytoluene BHT) are added. The test mixture's drop in absorbance is seen at 517 nm after 30 min. The assays are

performed three times. The following equation is used to get the antioxidant activity percentage (I%) for each sample:
I (%) = [(Absorbance of control- Absorbance of the sample)/Absorbance of control)]X100.

5.3. Reducing power assay

According to Oyaizu[22], the antioxidant capacity of olive leaves was evaluated. Each olive leaf extract was diluted in 500 µl of 1% phosphate buffer (pH 6.6), 200 µl of distilled water, and 2.5 ml of potassium ferricyanide solution K3Fe(CN)6 (1% w/v). For 20 min, the mixture was maintained at 50 °C. 2.5 ml of 10% w/v trichloro acetic acid were then added after cooling. To obtain an upper layer, the mixture was then centrifuged at 650 rpm for 10 min. Then, 500 µl of the supernatant layer was combined with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%, w/v). Ascorbic acid and the artificial antioxidant (BHT) served as benchmarks. Similar procedures were used to set up the control. With the aid of a spectrophotometer, the absorbance was measured at 700 nm. The results of a linear regression analysis were used to obtain $A_{0.5}$ values, which were then represented as (µg/ml).

6. Statistical Analysis

Triplicates of each experiment were run in each experiment. Means ± standard deviation are used to express the results. One-way ANOVA or the Student's test are used to assess the statistical differences. A p-value of 0.05 or lower qualifies a difference as statistically significant.

7. Results And Discussion

7.1. Physicochemical study

The data presented in Table (1) shows the physicochemical analysis (yield extraction, humidity, ash content and lipid) of leaves from ten Algerian olive cultivars. The findings of yield, humidity and ash percentages report here a very highly significant variation ($p<0.0001$) according to the variety, while humidity percentages vary significantly ($p<0.05$) according to this variable.
The highest extraction yield of the dry extract is found in Neb Djmel cultivar (404.00 ± 24.07 mg/g dry powder), while the lowest yield is recorded in Agraraz cultivar (16.83 ± 1.03 mg/g dry powder). Our values are higher than those found by Cho et al., [23].
Solvent extraction is the main method adopted by most researchers to extract antioxidant components from olive leaves, and repeated extraction steps accomplish the yield extraction.
The humidity percentage ranges from (37.86 ± 1.63 %) for Boughanfous to (46.75 ± 2.51%) for Takasrit cultivars. Results of Ibrahim et al., [18] in Greek olive leaves indicate higher levels than the ours (50.5%).
The analysis for the ash content find yields values ranging between (4.54 ± 0.13 and 13.03 ± 0.20%) in Tefah and Blanquette de Guelma cultivars, respectively. These values are higher than

those found by Diana Melo Ferreira et al., [24] in Portuguese varieties and closely related to those found by Ibrahim et al., [25].

Furthermore, the highest value of lipid content is detected in Tabelout variety (11.74 ± 0.24 %) and the lowest in Tefah variety (4.03 ± 0.16%). Results from this study outperform those from Fatmi et al., [26].

Table 1: Physicochemical analysis (yield extraction, humidity, ash content and lipid) of leaves from ten Algerian olive cultivars.

	Aaleh	*Agraraz*	*Aghechrem d'elBoussaar*	*Aghechrem de Tizeza*	*Boughanfous*	*Blanquette de Guelma*	*Neb Djmel*	*Tabelout*	*Takasrit*	*Tefah*
Yield extract (mg/g dry powder)	219.10 ± 16.09 f	168.30 ± 10.36 b	365.30 ± 8.24 a	320.30 ± 12.27 d	217.50 ± 17.23 fg	305.80 ± 2.15 h	404.00 ± 24.07 i	322.40 ± 12.48 de	277.90 ± 11.40 c	342.80 ± 35.40 i
Humidity (%)	45.01 ± 2.19 b	42.64 ± 2.48 b	45.31 ± 1.77 b	43.28 ± 3.70 b	37.86 ± 1,63 a	40.08 ± 1.79 a	41.00 ± 1.34 a	40.56 ± 2.49 a	46.75 ± 2.51 b	41.45 ± 3.74 b
Ash content (%)	4.56 ± 0.27 b	6.03 ± 0.26 cd	5.77 ± 0,34 cd	6.83 ± 0.07 e	7.70 ± 0.63 e	4.39 ± 0.40 a	6.42 ± 0.28 cd	13.03 ± 0.20 f	5.37 ± 0.37 c	4.54 ± 0.13 a
Lipid content (%)	5.05 ± 0.06 c	5.98 ± 0.18 d	4.32 ± 0.05 ab	11.18 ± 0.24 g	6.00 ± 0.10 de	4.28 ± 0.24 a	6.21 ± 0.39 de	11.74 ± 0.24 h	7.18 ± 0.33 f	4.03 ± 0.16 a

7.2. Pigments

The results of some pigments contents (dry basis) are listed in Table (2). The analysis of this parameter in the leaves shows a very highly significant differences ($p<0.0001$) between varieties regarding chlorophyll b, and carotenoids while no significant differences are found in the parameter of chlorophyll a. Indeed, results of Bahloul et al.,[27], indicate that cultivar influenced significantly contents of chlorophyll a and chlorophyll b but those of Edziry et al.,[28] negate any differences between varieties in terms of chlorophyll a or chlorophyll b.

The content of chlorophyll a and b contribute energy to the primary production[29], as well to evaluate the response of vegetation to: changing of environmental conditions, disease, nutritional and environmental stress [30].

The ethanol extracts of the leaves of Aaleh cultivar are characterized by the highest total of chlorophyll a (2827.96 ± 36.17 µg/g) and chlorophyll b (529.72 ± 32.41 µg/g) amounts, while Tabelout cultivar is characterized by the lowest amounts of chlorophyll a (764.52 ± 25.41 µg/g) and chlorophyll b (261.29 ± 34.50 µg/g). Therefore, our varieties are rich in chlorophyll compounds and the obtained values are higher than those found by Lorini et al., [31].

However, the chlorophyll a concentrations are higher than those of chlorophyll b in all studied cultivars which can be justified by the fact that the chlorophyll constitutes the primary pigment while chlorophyll b is a secondary pigment [32].

The carotenoids levels range between (20.66 ± 0.92 µg/g) and (44,13 ± 3,08 µg/g), respectively in Aelah and Boughanfous cultivars. Similarly, carotenoids were previously reported in Tunisian cultivars by Tarchoune et al., [33].

Carotenoids are antioxidants which exercise several health effects by contributing energy to the photosynthetic and photo protective system in plants [34].

In contrast, the whole of studied cultivars displays only trace amounts of lycopene. The important levels of chlorophyll and carotenoids in our studied leaves cultivars can be exploited to produce a natural food dye by incorporation of rich pigments of olive leaves.

Table 2: Pigment quantification of Algerian olive leaves extracts (*Olea europaea* L.) from ten cultivars. The results are repor ted as mean± SD (n=3)

	Aaleh	Agraraz	Aghechren d'ellousse	Aghechren de Titeste	Boughanfous	Blanquette de Guelma	Neb Djmel	Tabelout	Takasrit	Tefah
Chlorophyll a (µg/g)	2827.96 ± 36.17 e	1155 ± 15.30 de	939.85 ± 73.25 c	1084.09 ± 67.29 cd	866.42 ± 23.45 ab	938.3 ± 33.90 c	825.10 ± 37.43 a	764.52 ± 25.41 a	855.6 ± 50.34 ab	1169.93 ± 75.09 de
Chlorophyll b (µg/g)	529.72 ± 32.41 e	477.70 ± 21.86 de	369.15 ± 34.38 bc	249.38 ± 16.58 a	518.84 ± 18.84 de	474.23 ± 25.19 de	369.34 ± 16.80 bc	261.29 ± 34.50 a	332.74 ± 28.17 b	446.48 ± 27.49 d
Carotenoids (µg/g)	20.66 ± 0.92 a	22.97 ± 0.59 b	27.94 ± 0.06 d	30.22 ± 3.24 d	44.13 ± 3.08 d	37.2 ± 3.26 d	38.81 ± 4.98 d	33.83 ± 5.51 d	26.09 ± 3.04 bc	36.84 ± 2.80 d
Lycopène	0.03 ± 0.01 b	0.89 ± 0.05 e	0.20 ±0.01 d	1.30 ± 0.22 f	0.05 ± 0.00 bc	0.02 ± 0.00 a	2.92 ± 0.03 g	0.05 ± 0.00 bc	0.03 ± 0.00 b	0.01 ± 0.00 a

7.3. Phenolics, flavonoids and condensed tannins compounds

Total phenolics, total flavonoids and condensed tannins contents of ethanolic extracts leaves from ten Algerian olive cultivars are presented in Figure (1). The total phenolics contents are expressed as gallic acid equivalent, while flavonoids contents as quercetin equivalent. There is a very high statistically difference ($p<0.0001$) among different cultivars. The phenolics contents are found in the range of (206.70 ± 10.19 mg GAE/g DE to 322.90 ± 11.04 mg GAE/g DE). The flavonoid content is found in the range of (62.94 ± 2.46 mg QE/g DE) to 122.00 ± 8.40 mg GAE/g DE.

Our results express that Aeleh leaves extracts contain the highest amount of both phenolic and flavonoids, while Teffah leaves extracts contain the least amount of both phenolics and flavonoids compounds. Our polyphenolic contents are much higher than those reached by Nicolì et al.,[35].

Actually, the polyphenolic compounds occupy the most attention in the world due to their protection of our health against antioxidant activities. As well as, the amount of the total phenols content varies according to many factors mostly to genotype of olive tree [36].

The flavonoids compounds constitute the most important group of olive leaves polyphenols. Several health benefits are linked to the presence of flavonoids in our nutrition in reason of their ability to reduce stress activity [37]. Our findings are much higher than those of Mansour et al.,[38], but less than those found by Lfitat et al.,[39]. In fact, flavonoids concentrations depend on many factors including: genetic factors [40].

The results in figure 01 also show that the amounts of total phenolics compounds in our ethanolic extracts present the same trends profile as for flavonoid content among studied varieties.

In vitro, experimental system has also shown that flavonoids possess anti-inflammatory, anti-allergic, antiviral and anti-carcinogenic properties [41]. The primary therapeutic characteristics associated to tannins are provided by condensed tannins, also referred to as proanthocyanidins, which are secondary metabolites of plants [42]. The maximum amount of condensed tannins is 7.18 ± 0.14 mg CE/g DE in Neb Djmel variety. Similarly, Fatmi et al., [26] described the presence of these compounds in Algerian olive leaves but with contents lesser than of ours (6.34 mg CE/g DE). Dhull et al .,[43] has signaled that the presence of condensed tannins in extracts is not to be neglected. These substances provide natural food formulations made from tannin-rich plants, a distinctive flavor as well as antioxidant, anti-carcinogenic, and cardio protective effects [44].

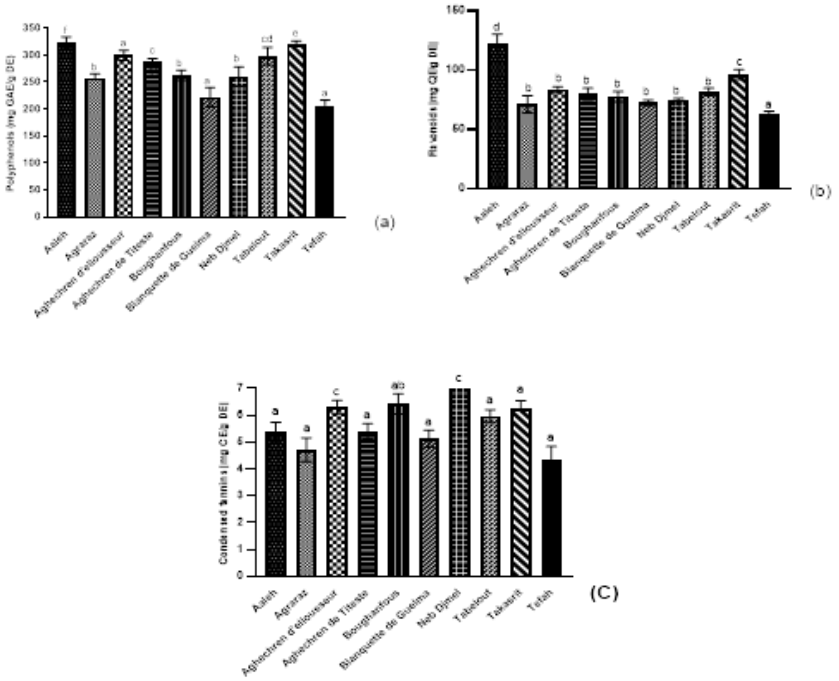

Fig.1: Polyphenols (a), flavonoids (b), condensed tannins (c) of Algerian olive leaves extracts (*Olea europaea* L.) from ten cultivars. The results are reported per 1 g of extract and are presented as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$). DE: dry extract, GAE: Gallic acid equivalents, CE: Catechin equivalent.

7.4. Antioxidant activity

The total antioxidant capacity (TAC), free radical DPPH, and reducing power tests were used to measure the antioxidant properties of olive leaf extracts.

7.4.1. DPPH scavenging activity

DPPH is a stable nitrogen-centred free radical. The process of hydrogen or electron-donation capacity of antioxidants is measured by DPPH assay in which the color changes from violet to yellow.

Figure (2) shows the results of the 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity of various ethanolic extracts of olive leaves and synthetic antioxidant BHT. According to the results, the ethanolic olive leaves extracts show a considerable scavenging activity ranging between 55.46

± 2.56 and 72.21 ± 1.35 µg/ml. The DPPH scavenging activities are in the order: Blanquette de Guelma > Agraraz > Tefah > Aghechren d'ellousseur > Neb Djmel > Aghechren de Titeste > Takasrit > Tabelout > Boughanfous > Aaleh. Very high significant differences are detected between our olive leaves extracts ($p<0.0001$).

Comparing our results to those recorded by Mansour et al., 38 with values ranging between 48 and 56 µg/mL and lower than those of Nashwa et al.,[45] with value of 83 µg/ml. A higher IC 50 value indicates a low antioxidant activity.

As a matter of fact, the antioxidant activity in our study is very high and usually explained by the rich phenolics and flavonoids contents that are extracted. Due to the presence of phenolic OH groups in their structures, such as hydroxytyrosol and oleuropein, phenols have demonstrated high DPPH radical scavenging action in the past. By donating a hydrogen atom to break the free radical chain and transform them into more stable molecules, they operate as reductones and may be markers of the compound's strong antioxidant activity [46]. Additionally, the free radical scavenging activity is proven in a DPPH system, where it is found that our living leaf extracts have stronger radical scavenging activity ($p<0.05$) than the synthetic antioxidants BHT and ascorbic acid 45. Phenolic compounds demonstrate major role for antioxidant activity of olive leaves which is attributed assessed individually or by the synergetic effect between them [47].

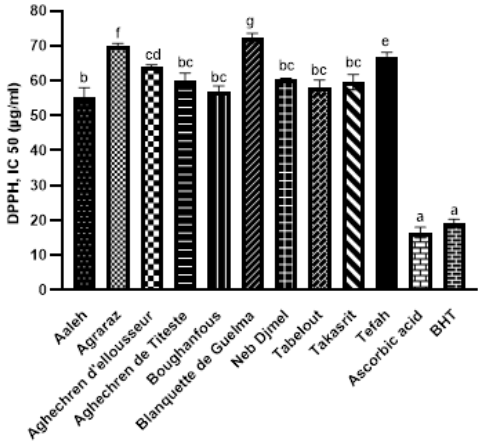

Fig.2: Free radical scavenging activity of ethanolic extracts (*Olea europaea* L.) from ten Algerian olive leaves cultivars by the DPPH assay. . Values are expressed as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$).

7.4.2. Total antioxidant capacity

The total antioxidant capacity assay quantifies a sample's capacity to neutralize a free radical by supplying it with an electron [22]. The results are shown in Figure (3) and are expressed as ascorbic acid equivalent. Under acidic circumstances, Mo (VI) is converted to Mo (V) and phosphate Mo (V) complex is formed, which are characteristics of the total antioxidant capability. There is a very highly statistically significant difference ($p<0.0001$) among the antioxidant activity of all cultivars. The antioxidant potential of all extracts, measured in mg ascorbic acid equivalents, can be graded

as follows: Blanquette de Guelma < Agraraz <Tefah < Aghechren d'ellousseur < Aghechren de Titeste < Neb Djmel < Takasrit < Tabelout < Boughanfous < Aaleh.
Compared to other extracts, the TAC assay results show that the ethanolic extract of the Aaleh cultivar has the highest total antioxidant capacity (349.80 ±14.00 mg AAE/g DW), while the Tefah cultivar has the lowest (270.73 ± 4.53 mg AAE/g DW). Our results are lower than those recorded by Lfitat et al., [39] with values ranging between 335.9 ± 6.04 and 407.8 ± 8.20 mg AAE/g DW.

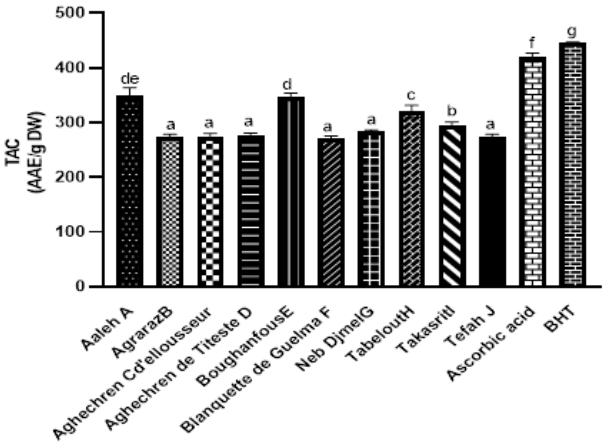

Fig. 3: Total antioxidant capacity of ethanolic extracts (*Olea europaea* L.) from ten Algerian olive leaves cultivars. Values are expressed as mean± SD (n=3). Columns marked with different letters are statistically different (p < 0.05). AAE/g DW: acid ascorbic equivalent/ g dry weight.

7.4.3. Reducing Power

Figure (4) shows the reducing power of ethanolic olive leaves extracts compared with BHT and ascorbic acid. $A_{0.5}$ values—the effective concentration at which the absorbance is 0.5 for decreasing power—are used to express the results. In the presence of antioxidants, reductones reduce the Fe^{3+}/ferricyanide complex to the ferrous form[48]. The strength of the solution's green/blue color serves as a gauge. The investigated extracts display potent antioxidant power with $A_{0.5}$ values ranging between 49.96 ± 0.27 and 53.95 ± 0.73 µg/ml in the following order: Blanquette de Guelma > Agraraz > Aghechren d'ellousseur > Tefah > Aghechren de Titeste >Neb Djmel > Takasrit >Tabelout > Boughanfous >Aaleh. The high reducing power of these extracts indicates their high antioxidant activity.
The reducing power shows high significant differences between all ethanolic extracts ($p<0.0001$). $A_{0.5}$ of reducing power of ascorbic acid are found to be lower than the studied ethanolic extracts (11.23 ± 0.09 µg/ml) while the BHT is found to be higher (98.38 ± 0.65 µg/ml (Figure 3). These results are similar to those reported by Mansour et al,.[38] who have found the values of reducing power ranging between 51.21 and 55.16 µg/ml comparing to ascorbic acid as a standard ($A_{0.5}$= 15.57 µg/ml). On the other hand, these values are very higher than those described by Nashwa et al., [39] who has found a value of 2.45 µg/ml compared to BHT as a standard (A0.5= 5.7 µg/ml). According to reports[49], the reducing power of olive leaves can be explained by the effectiveness of

phenolic and flavonoid components that could combine with free radicals to stabilize and prevent radical chain reaction.

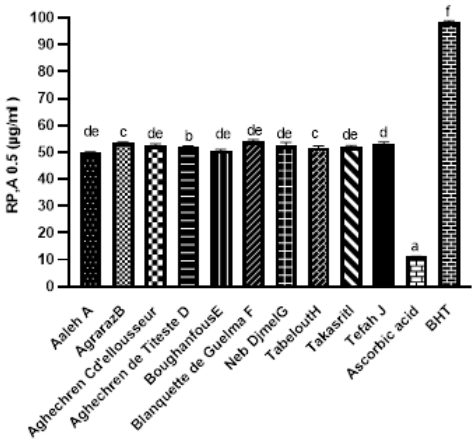

Fig. 4: Reducing power of ethanolic extracts (*Olea europaea* L.) from ten Algerian olive leaves cultivars. Values are expressedas mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$).

Conclusion

On the basis of the overhead results, it can be concluded that Algerian olive leaves are a rich source of the valuable pigments, antioxidants especially carotenoids, polyphenolic including flavonoid compounds and condensed tannin compounds. Consequently, complementary techniques (total antioxidant capacity, DPPH free radical scavenging activity, and reducing power) have demonstrated the extracts excellent antioxidant properties. Ultimately, the information gathered from this study confirms earlier research on the bioactive chemical richness of olive leaves and may be used as a natural source of antioxidants for food additives, nutritional supplements, cosmetics, pharmaceuticals, and nutraceuticals.

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