Original Article

Quantification And Antioxidant Activity Of Olive Leaves (Olea Europaea L.) Of Algerian Varieties

Mokhtar Guissous*[1], Hasna Boulkroune[1] Nasreddine Mekhoukh[2]

[1] Department of biology, Faculty of Natural and Life Sciences and Earth and Universe Sciences. University Mohamed El Bachir El Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.

[2] Department of Physico-Chemical Biology, Faculty of Nature and Life. University Abderrahmane Mira-Bejaia, 06000 Algeria.

m.guissous@univ-bba.dz

Received : 11/01/2023 ; Accepted : 25/05/2023

DOI: 10.47750/pnr.2023.14.04.98

Abstract

Over the past decade, there has been a growing interest in research focused on extracting biologically active compounds from natural sources as Olea europaea L. The objective of the current study was to explore the content of some antioxidants extracted from olive leaves of nine Algerian cultivars. Subsequently, the antioxidant activities of these compounds extracts were assessed.

The antioxidant properties were determined through total antioxidant capacity (TAC), DPPH, and reducing power assays. The results indicated that ethanolic extracts of all studied cultivars are rich in total antioxidant compounds. Abani extract exhibited the highest TAC (499.70 ± 15.93 mg AAE/g DW), the highest reducing power (A0.5 = 11.03 ± 1.47 µg /ml) and was the most effective against the DPPH free radical (IC50 = 46.67 ± 1.58 µg /ml). Furthermore, the study revealed that the cultivar factor significantly influenced the antioxidant properties. The results obtained in the present study indicate that ethanolic extract of Algerian olive leaves constitute a potential source of natural antioxidants, and support the traditional use of this plant in the treatment of infectious diseases.

Keywords: Olea europaea L., antioxidant, TAC, DPPH, reducing power.

INTRODUCTION

The Algerian flora is rich in medicinal plants including olive plant. The olive tree (Olea europaea L.) is of great significance, being one of the major fruit cultivated trees. It holds considerable economic and social value, and its diverse byproducts, including its leaves, have the potential to provide a wide range of benefits that are commonly used in folk medicine for the treatment of several diseases.

The beneficial effects of these leaves are mainly due to the presence of bioactive compounds, especially secondary metabolites such as phenolic, carotenoid and flavonoid compounds. Some evidence suggested that the biological actions of these compounds are related to their antioxidant activity against free radicals **(Gryglewski et al., 1987)** which contribute to more than one hundred disorders in humans **(Kumpulainen & Salonen, 1999).**

Indeed, olive leaves extracts have shown efficacy as antioxidants **(Ben Salem et al., 2015)**, anti-inflammatory **(Magrone et al., 2018)**, hypoglycemic properties **(Somova et al., 2004)** and anti-hypertensive activity **(Paiva-Martins et al., 2014)**. Furthermore, several studies have shed light on the antimicrobial activity of olive leaf extracts **(Korukluoglu et al., 2008).**

Thus, olive leaves can be utilized in various applications such as food preservation, cosmetics, pharmaceutical products, alternative medicine, and natural therapies **(Andersen & Markham, 2005)**.

Unfortunately, in Algeria few studies on olive leaves have been undertaken. Our interest in these products comes from these observations and the present work consists in filling the lack of information about the properties of the leaves of some Algerian olive cultivars.

The present study was designed to investigate the total antioxidant contents, as well as the antioxidant activities of leaves ethanolic extracts from nine Algerian cultivars by using three different methods including: DPPH, free radical scavenging and ferric reducing power assays.

MATERIALS AND METHODS

1. Plant material

The olive leaves utilized as plant material were manually harvested from nine different cultivars of Olea europaea L. Abani, Aghenfas, Aguenaou, Aimel, Bouchouk Lafayette, Ferkani, Hamra, Limli, Longue de Miliana. The collection took place in March during the 2021/2022 crop season from mature trees at the experimental station of the Technical Institute of Fruit and Vine Arboriculture (ITAFV) in TAKRIT, located in the city of Bejaïa, (estnorthen Algeria). Three replicates were taken from each collected sample, cleaned, and dried at 25°C in a shaded area for 15 days. The dried leaves were then powdered using an electric grinder and sieved to obtain a fine powder. The resulting powder was stored in a dry and dark place until extraction and analysis.

2. Preparation of plant extracts

Using a magnetic stirrer and 150 ml of 70% ethanol, 10g of powdered olive leaves undergo extraction for a duration of 3 hours at room temperature and in the absence of light. Following the completion of the extraction process for various cultivars of Olea europaea L. leaves, the extracts were subjected to filtration through microfilters to obtain a clear crude extract solution. Subsequently, the separation procedure was reiterated, and the solvent was evaporated under vacuum conditions at 40°C using a rotary evaporator. All extraction processes were conducted in triplicate. The resulting extracts were then frozen at -4°C for subsequent analysis. The extraction yield (%) was calculated as the ratio of the amount of dry extract obtained to the initial amount of powder used for the extraction process.

3. Phytochemical study

3.1. Determination of total phenolic compounds

The total phenolic content in the ethanol extracts of Olea Europaea L. leaves was determined using the Folin Ciocalteu reagent method, as described by **(Singleton & Rossi, 1965).** In this method, 200 µl of the sample or standard, appropriately diluted, was added to 1 ml of Folin's reagent that has been diluted 10 times. After 4 minutes, 800 µl of a 7.5% sodium carbonate solution was added. The mixture is allowed to react for two hours at room temperature in the dark. The acquired data were analyzed using the linear regression equation of the end acid calibration graph: $Y= 0.0068 x + 0.056$. $R^2= 0.9991$, where Y represents absorption intensity and X represents total phenolic compounds expressed as gallic acid equivalent (mg GAE/g DE). The R2 value is 0.9991.

A Shimadzu™ UV-VIS 1800 Spectrophotometer, a double-beam UV-Vis spectrophotometer from the USA, is employed to measure the absorbance of the solution and the blank at 760 nm. Three measurements were taken again to ensure accuracy.

3.2. Determination of total flavonoids

The total flavonoids present in the leaf extracts are estimated using the method developed by **Jain et al (2011)**. In this method, 1 ml of 2% $AlCl_3$ was added to 1 ml of each extract or standard solution at various concentrations. After 1 hour at room temperature, the absorption at 430 nm was read using a double-beam UV-vis spectrophotometer, such as the Shimadzu™ UV-VIS 1800 Spectrophotometer USA.

To create the classic quercetin calibration curve, 1 ml of quercetin at various concentrations was added. Triplicate samples were used for each analysis. The total flavonoid levels were determined in milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE) using the following equation based on the calibration curve: $y = 0.333 + 0.024$. Where, y is the absorbance and x is the quercetin content ($\mu.ml^{-1}$). The coefficient of determination (R2) for the calibration curve is 0.9917.

3.3. Antioxidant activity

3.3.1. Total antioxidant capacity (TAC)

The method described by **Prieto et al (1999)** was employed to assess the total antioxidant capacity of the extracts. An aliquot of 0.3 ml from each ethanolic extract (1 mg/ml) was combined with 3 ml of the reaction solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate). The tubes were then incubated for 90 minutes at 95 °C. After the mixture has reached

room temperature, the absorbance at 695 nm was measured against a blank solution using the Shimadzu™ UV-VIS 1800 Spectrophotometer, USA.

The total antioxidant capacity was calculated using the equation derived from a standard ascorbic acid calibration curve. The results were expressed as milligrams of ascorbic acid equivalents per gram of dry extract.

3.3.2. DPPH free radical scavenging activity

Following the procedure outlined by **Burits & Bucar (2000)**, the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was employed to evaluate the free radical scavenging activity of extracts. In summary, a stock solution of DPPH was prepared by dissolving it in 100 ml of methanol, and then it was diluted to achieve an absorbance of 0.98 ± 0.04 at 517 nm. The control, without the test sample, was infused with an equivalent 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) is added. The decrease in absorbance of the test mixture is observed at 517 nm after 30 minutes. The assays are conducted three times. The antioxidant activity percentage (I%) for each sample is calculated using the following equation:

%=[(Absorbance of controlAbsorbance of control−Absorbance of the sample)×100].

3.3.3. Ferric Reducing Antioxidant Power (FRAP)

FRAP analysis was conducted following the protocol outlined by **Oyaïzu (1986)**. In this procedure, extracts (200 µL) were combined with phosphate buffer (500 µL, 2.0 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1.0%), and the mixture was incubated at 50°C for 20 minutes. Subsequently, trichloroacetic acid (10%) in a volume of 2.5 mL was added to the mixture, which was then subjected to centrifugation at 650 rpm for 10 minutes. The upper layer of the solution (500 µL) was then mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture indicated an increase in reducing power.

4. STATISTICAL ANALYSIS

Three replicates were conducted for each experiment. The results were presented as means ± standard deviation. Statistical differences were assessed using either one-way ANOVA or the Student's t-test. A p-value of 0.05 or less was considered indicative of a statistically significant difference.

5. RESULTS AND DISCUSSION

5.1. Extraction yields

The extract yields of the various olive leaf cultivars were expressed as percentages (%) and were presented in Figure (1). The obtained yields varied significantly ($P < 0.05$) from 14.60 ± 0.96 % in Aghenfas cultivar to 36.45 ± 1.59 % in Abani cultivar.

In terms of varietal distinctions, the yields of ethanolic extracts were observed in the following order: Abani> Longue de Miliana> Hamra > Aguenaou > Aimel > Limli> Ferkani > Bouchouk Lafayette >Aghenfas. Overall, the obtained yield was comparatively higher than the results reported by **Medfai et al. (2020)**, who detailed extraction yields of hydroethanolic extracts (30:80; v/v) from Tunisian cultivars, ranging from 24.5% to 32.8%. Various factors, including the particle size of the plant material under investigation and the solvent's polarity, contributed to the influence on the extraction yield. Additionally, factors such as storage conditions, duration, harvest period, as well as the selected extraction method and conditions, played pivotal roles in shaping the overall yield **(Ali haimoud, 2017).**

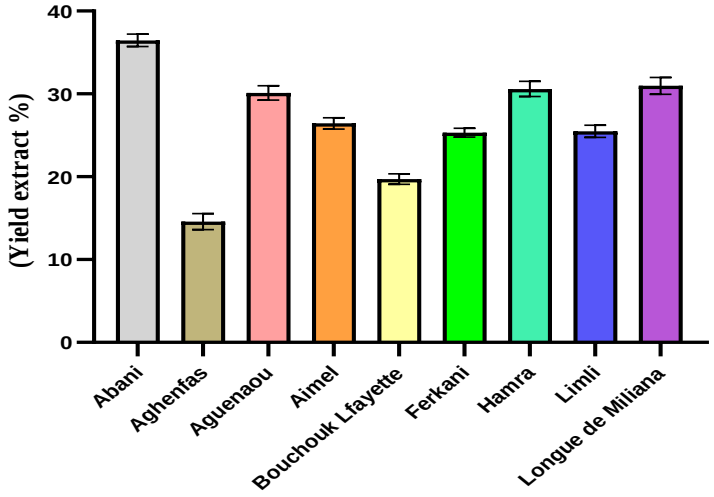

Figure 1. Yield extracts of Algerian olive leaves (Olea europaea L.) from nine cultivars. The results are reported as mean± SD (n=3).

5.2. Total phenolic content

The results of the antioxidant quantification of the various extracts utilized in this study, as outlined in figure (2A), exhibited a broad range of values dependent on the olive variety employed. Notably, the ethanolic extracts of the Abani variety demonstrated the highest polyphenol contents, registering approximately 496.35 ± 16.59 mg GAE/g DE. Conversely, the ethanolic extracts of the Aghenfas variety displayed lower polyphenol contents (160.40 ± 14.36 mg GAE/g DE) compared to the rest of the cultivars. The findings of phenolic content reported a highly significant variation ($p<0.001$) according to the variety.

The results are much higher than those reported by **Salah et al. (2012)** on Gerboui variety from Tunis (142.21 ± 3.53 mg GAE/g DE) and **da Rosa et al. (2019)** on Arbequina cultivar (from Brazil) ranging from 98.14 to 115.75 mg GAE/g DM.

Phenols play a crucial role as essential constituents in plants. The strong positive correlation between total phenols and the antioxidant activity observed in many plant species can be attributed to the scavenging ability of their hydroxyl groups, as highlighted by **Vinson et al.** in **1998**. Additionally, the efficacy of phenolic compounds as effective hydrogen donors further underscores their role as potent antioxidants, as reported by **Yen et al.** in **1993.**

5.3. Total flavonoid content

Flavonoids constitute a highly varied category of phenolic compounds found in olive leaves **(Oliveira et al, 2021)**. Derived from plants, flavonoids exhibit robust antioxidant, antimicrobial, and anti-hyperglycemic properties owing to their capability to neutralize free radicals, combat various bacterial strains, and hinder starch-digesting enzymes **(Aron & Kennedy, 2008).**

According to the results figure (2B), the flavonoid contents of ethanolic extracts of studied varieties exhibited very significantly ($P< 0.01$) variation according to the variety, averaging from 125.73 ± 3.34 to 242.5 ± 4.32 mg QE/g DE, in Aguenaou and Aghenfas cultivars respectively. In comparison, the literature reported lower flavonoid contents in Brazilian (19.4 ± 1.3 mg QE/g DW) **(Lins et al., 2018)** and Tunisian olive leaves **(Sifaoui et al., 2014)** (19.25 ± 0.07 to 35.16 ± 0.00 QE/g DE).

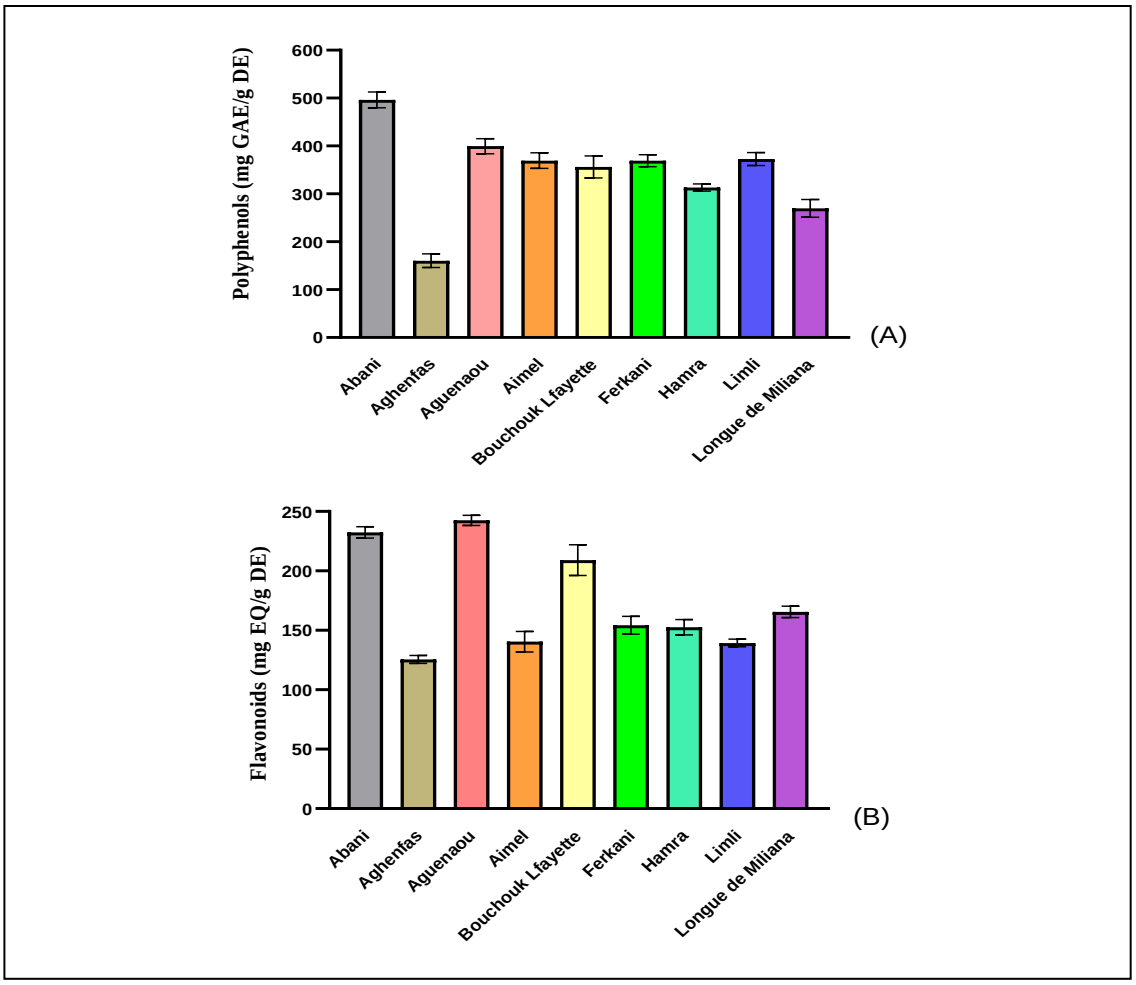

Figure 2: Polyphenols (A), flavonoids (B), of Algerian olive leaves extracts (Olea europaea L.) from nine cultivars. The results are reported per 1 g of extract and are presented as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$). DE: dry extract, GAE: Gallic acid equivalents, CE: Catechin equivalent.

5.4. Antioxidant activity

The assessment of antioxidant activity was conducted through three widely employed assays: total antioxidant capacity (TAC), free radical DPPH, and reducing power tests **(Mokrani & Madani, 2016)**. These methods rely on the capacity of antioxidants to neutralize free radicals **(Marc et al, 2004).**

5.4.1. DPPH scavenging activity

The DPPH model offers a quicker assessment of antioxidant activity compared to alternative methods. According to the DPPH scavenging activity presented in figure (3A), the ethanolic extract of Abani variety represented the most active extract with an IC50 of about 46.67 ± 1.58 µg /ml and it is significantly ($P < 0.05$) higher than values found in other olive leaf cultivars. On the other hand, the IC50 of the ethanolic extract of the Aghenfas variety (84.67 ± 1.75 µg/ml) was significantly ($P < 0.05$) lower than those found for the other extracts tested. However, the order of reactivity of the rest cultivars in ascending order: Longue de Miliana< Aguenaou< Hamra< Ferkani < Bouchouk Lafayette < Aimel < Limli.

Furthermore, all extracts tested exhibited lower antioxidant activity than, ascorbic acid (11.23± 2.65 µg /ml) and BHT (IC50 = 15.28 ± 2.65 µg /ml).

A comparison of our findings with those documented in the literature revealed variations depending on the respective studies. The outcomes of our study demonstrated higher antioxidant activity compared to those reported by **Abdul et al. (2015)**. They identified IC50 values of 196 mg/l and 82 mg/l using the DPPH method, for aqueous extracts of olive leaves of Frantio and Mission, respectively from Pakistan. The high DPPH radical scavenging activity of these cultivars suggests their use in diseases arising from free radical attack.

Moreover, a study by **Silva et al. (2010a)** examined Brazilian olive varieties, including Arbequina and Koroneiki. The IC50 values reported for the DPPH assay in their study exhibited variations similar to our results, reinforcing the notion that the antioxidant capacity of olive leaf extracts is inherently tied to the specific cultivar under investigation.

5.4.2. Ferric Reducing Antioxidant Power

In addition to the widely used DPPH assay, the ferric reducing antioxidant power assay is another important method employed to assess the antioxidant capacity of biological samples, including plant extracts such as those from olive leaves. Antioxidants can be described as substances in the sample that act as reductants, leading to the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Consequently, the presence of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm **(Chung et al, 2002)**. As the absorbance increases, the reducing power proportionally rises **(Jayaprakasha et al, 2001)**

Similarly, the examined extracts demonstrate robust antioxidant potency, with $A_{0.5}$ values ranging from 11.03 ± 1.47 to 44.01 ± 2.93 µg/ml in the following sequence: Abani< Longue de Miliana<Aguenaou<Hamra<Ferkani<Aimel < Limli < Bouchouk Lafayette <Aghenfas. There are highly significant differences in reducing power among all ethanolic extracts ($p<0.0001$). The elevated reducing power observed in these extracts serves as an indication of their remarkable antioxidant activity.

The $A_{0.5}$ values for the reducing power of ascorbic acid were found to be lower (9.68± 1.65 µg/ml) than those observed for the studied ethanolic extracts. In contrast, BHT exhibited higher reducing power (98.38 ± 0.65 µg/ml) (Figure 3B). These findings align with those reported by **Vladimir-Knezevic et al (2011)**. A higher absorbance in the reaction mixture signifies a greater reductive potential. The reducing capacity of a compound can be a significant indicator of its potential antioxidant activity indicating the substantial antioxidant activity of the examined extract **(Jayanthiand, 2011)**.

Total antioxidant capacity

The Total Antioxidant Capacity (TAC) assay is a widely used method to evaluate the overall antioxidant potential of a sample. This assay quantifies the sample's ability to neutralize free radicals by measuring its capacity to donate electrons **(Prior & Schaich, ,2005)**. The principle behind TAC assays often involves the reduction of a colored complex, such as the conversion of Mo (VI) to Mo (V) under acidic conditions, leading to the formation of a phosphate Mo (V) complex. This transformation is indicative of the sample's total antioxidant capability.

The results of the phytochemical composition of the various extracts used in this study, as presented in figure (3C), showed a wide range of variation depending on variety. However, the order of their reactivity was: Abani> Longue de Miliana>Aguenaou>Hamra>Ferkani > Bouchouk Lafayette > Limli > Aimel >Aghenfas.

By comparing different studied cultivars, a highly significant statistical difference ($p<0.0001$) was reported among the antioxidant activities of various extracts, demonstrating the sensitivity and discriminative power of the TAC assay.

As observed, Abani ethanolic extract displayed the highest antioxidant capacity (499.70 ± 15.93 mg AAE/g DW), while Aghenfas cultivar displayed the lowest (179.50 ± 3.71 mg AAE/g DW). Our results are higher than those recorded by **Abdul et al (2015).**

The antioxidant activity observed in Olea europaea L. is likely attributed to its polyphenols, flavonoids content. They acte as scavengers for various oxidizing species, including superoxide anion ($O2^{\bullet-}$), hydroxyl radicals, or peroxy radicals. Additionally, they serve as quenchers of singlet oxygen, as demonstrated by **Das & Ratty in 1986**. Many plant constituents have demonstrated free radical scavenging or antioxidant activity, as reported by **Aruoma** & **Cuppett in 1997**. Phenols, considered essential plant constituents, exhibit a highly positive correlation with the antioxidant activity of numerous plant species due to the scavenging ability of their hydroxyl groups, as indicated by **Vinson et al.** in **1998**. Furthermore, it has been

documented that phenolic compounds function as effective hydrogen donors, underscoring their role as potent antioxidants, as highlighted by **Yen et al.** in **1993**.

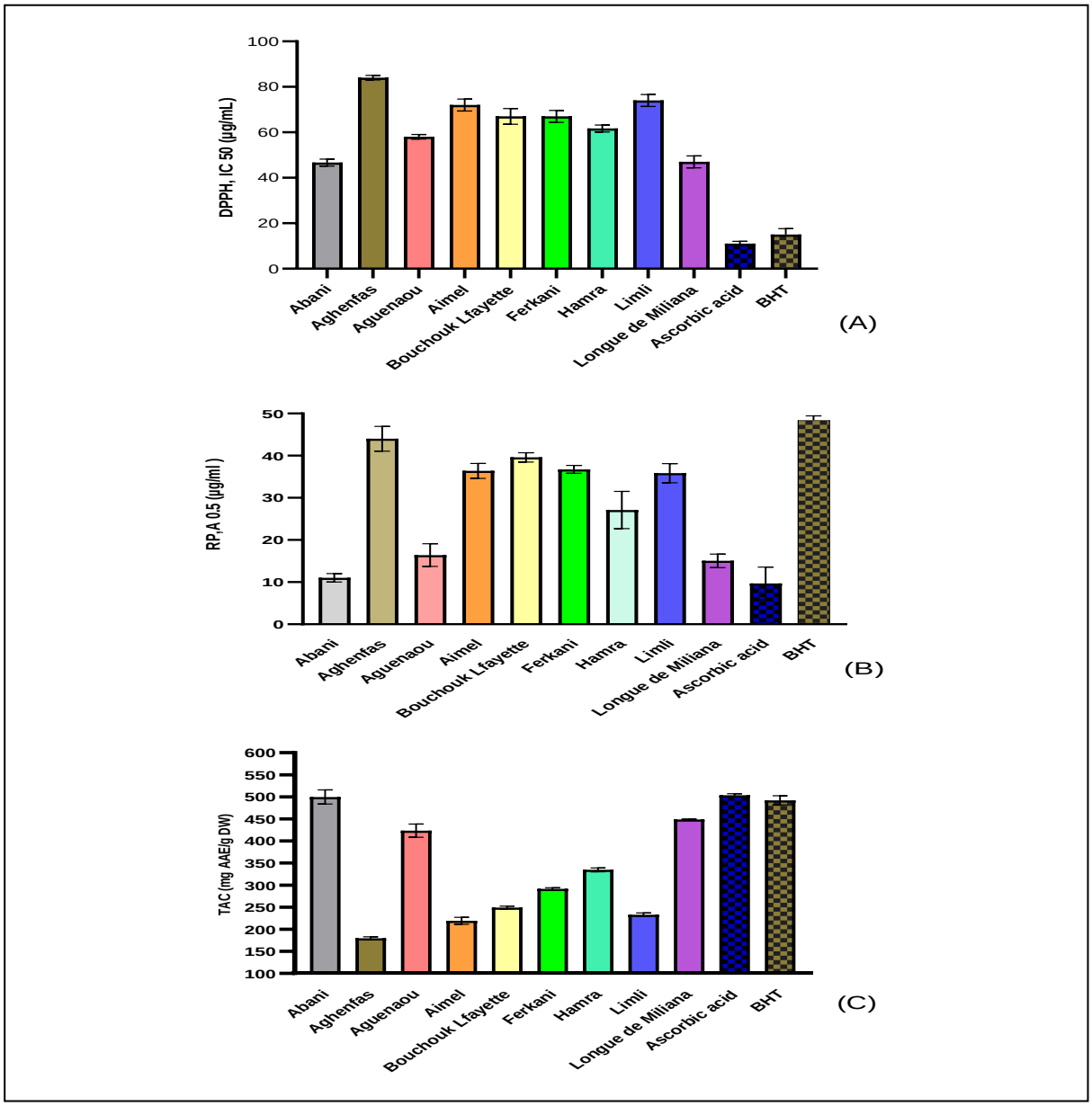

Figure 3. Free radical scavenging activity by the DPPH assay(A), Reducing power (B), Total antioxidant capacity (C) of ethanolic extracts (Olea europaea L.) from nine Algerian olive leaves cultivars. Values are expressed as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$).

CONCLUSION

Based on the above findings, it can be inferred that Algerian olive leaves are abundant in valuable pigments, particularly antioxidants such as carotenoids, as well as polyphenolic compounds including flavonoids and condensed tannins. Consequently, supplementary assays, including total antioxidant capacity, DPPH free radical scavenging activity, and reducing power, have demonstrated the excellent antioxidant properties of the extracts. In conclusion, the data gathered from this study substantiates previous research on the bioactive chemical richness of olive leaves. Additional research is necessary to pinpoint the precise active components responsible for antioxidant activities, as well as to isolate and identify the antimicrobial activity of these compounds.

REFERENCES

1. Abdul K, Syed Mubashar S, Syed Dilnawaz A, Aline Augusti B, Margareth Linde A, Abdul J, Imtiaz Q & Asmatullah K. Antioxidant activities and phenolic composition of Olive (Olea europaea) leaves. Journal of Applied Botany and Food Quality. 2015; 88, 16 - 21.
2. Ali haimoud S. Etude phytochimique et rôles biologiques des variétés des Phoenix dactylifera (datte) de l'Algérie, Thesis, ALLEM RACHIDA, 2017.
3. Andersen O.M & Markham K.R, Flavonoids: Chemistry, Biochemistry and Applications; CRC Press: Boca Raton, FL, USA, 2005.
4. Aron P M & Kennedy J A, Flavan-3-ols: Nature, occurrence and biological activity. Mol. Nutr. Food Res. 2008; 52, 79-104.
5. Aruoma OI & Cuppett SL. Antioxidant methodology In vivo and In vitro concepts.AOCS press, Champaign, Illinois.1997; pp.41-172.
6. Ben Salem M, Affes H., Ksouda K, Sahnoun Z, Zeghal K.M & Hammami S. Pharmacological activities of Olea europaea leaves. J. Food Process. Preserv. 2015; 39, 3128-3136.
7. Burits M & Bucar F. Antioxidant activity of Nigella sativa. Phytother Res.2000; 14, 323-328.
8. Chung Y C, Chang, C T, Chao W W, Lin C F & Chou, S T. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J Agric Food Chem. 2002; 50, 2454– 2458.
9. Das NP & Ratty AK. Effect of flavonoids on induced non- enzymatic lipid peroxidation. In: Cody V, Middleton E and Harborne J (eds.). Plant flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships. Liss AR, New York, pp.243-247,1986.
10. Da Rosa G, Vanga S K, Gariepy Y & Raghavan V. Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (Olea europaea L.), Innovat. Food Sci. Emerg. Technol. 2019; 58, 102234.
11. Gryglewski R J, Korbut R & Robak J. On the mechanism of antithrombotic action of flavonoids. Biochemical Pharmacol.1987; 36: 317- 321.
12. Jain D P, Pancholi S S & R. Patel. Synergistic antioxidant activity of green tea with some herbs", J. Adv. Pharm. Technol. Res., 2(3), 177-83 (2011).
13. Jayaprakasha G.K, Singh R P & Sakariah K K. Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro, Food Chem; 2001; 73, 285–290.
14. Jayanthiand P, Lalitha P. Reducing power of the solvent extracts of Eichhornia crassipes (Mart.) Solms. Int J Pharm Pharm Sci. 2011;3, Suppl 3, 126-128.
15. Korukluoglu M, Sahan Y & Yigit A. Antifungal properties of olive leaf extracts and their phenolic compounds, J. Food Saf. 2008; 28, 76-87.
16. Kumpulainen JT & Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry, UK. pp 178- 187, 1999.
17. Lins P G, Marina S, Piccoli Pugine A, Scatolini M & de Melo M P. In vitro antioxidant activity of olive leaf extract (Olea europaea L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon; 2018; (4), e0080.
18. Magrone T, Spagnoletta A, Salvatore R, Magrone M, Dentamaro F, Russo M A, Difonzo G, Summo C, Caponio F & Jirillo E. Olive Leaf Extracts Act as Modulators of the Human Immune Response, Endocr., Metab. Immune Disord: Drug Targets,18 , 85-93, 2018.
19. Marc F, Davin A, Deglene-Benbrahim L, Ferrand C, Baccaunaud M & Fritsch P. Méthodes d'évaluation du potentiel antioxydant dans les aliments, Med. Sci. 20 (2004) 458-463.
20. Medfai W, del M. Contreras M, Lama-Mun˜oz A, Mhamdi R, Oueslati I, Castro E. How cultivar and extraction conditions affect antioxidants type and extractability for olive leaves valorization, ACS Sustain. Chem. Eng. 8 (2020).
21. Mokrani A & Madani K. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit, Separation and Purification Technology. 2016; 162 68–76.
22. Oliveira A L S, Gondim S, Gómez-García R, Ribeiro T & Pintado M. Olive leaf phenolic extract from two Portuguese cultivars-bioactivities for potential food and cosmetic application. J. Environ. Chem. Eng. 2021; 9, 106175.
23. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr.1986; 44, 307-315.
24. Paiva-Martins F, Barbosa S, Silva M, Monteiro D, Pinheiro V. Mourão J L, Fernandes J, Rocha S, Belo L & Santos-Silva A. The effect of olive leaf supplementation on the constituents of blood and oxidative stability of red blood cells, J. Funct. Foods. 2014; 9, 271-279.
25. Prieto M, Pineda & M. Aguilar. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E, Anal. Biochem. 1999. 269, 337-341
26. Prior R L, Wu X & Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry.2005; 53 (10), 4290-4302.
27. Salah M, Abdelmelek H & Abderrabba M. Study of phenolic composition and biological activities assessment of olive leaves from different varieties grown in Tunisia, Med. Chem. 2012; 2 107–111.
28. Sifaoui I, Lopez-Arencibia A, Martín-Navarro C.M, Chammem , Reyes-Batlle M, Mejri M, Lorenzo-Morales J, Abderabba M & Pinero J E. Activity of olive leaf extracts against the promastigote stage of Leishmania species and their correlation with the antioxidant activity, Exp. Parasitol. 2014; 141 106–111.
29. Silva M L C, Costa R S, Santana A S, & Koblitz M G B. Compostos fenólicos, carotenoides e atividade antioxidante em produtos vegetais. Semina: CiênciasAgrárias. 2010a; 31(3), p. 669-682.
30. Singleton V & Rossi J. Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents, AJEV. 1965;16, 144-158.
31. Somova L I, Shode F O & Mipando M. Cardiotonic & antidysrhythmic effects of Oleanolic and ursolic acids, methyl maslinate and uvaol, Phytomedicine. 2004; 11, 121- 129.
32. Vinson JA, Yong H, Xuchui S & Zubik L. Phenol antioxidant quantity and quality in foods: vegetables. 1998; J. Agric. Food Chem, 46: 3630-3634.
33. Vladimir-Knezevic S, Blazekovic B, Stefan M.B, Alegro A, Koszegi T & Petrik J. Antioxidant activities and polyphenolic contents of three selected Micromeria species from Croatia, Molecules .2011; 16, 1454-1470.
34. Yen GC, Duh PD & Tsai CL. The relationship between antioxidant activity and maturity of peanut hulls. 1993; J. Agric. Food Chem., 41: 67-70.