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ANTIOXIDANT ACTIVITY AND PROTECTIVE EFFECT OF THE PHENOLIC FRACTION FROM OLIVE OIL AGAINST LIPID PEROXIDATION INDUCED BY H_2O_2 AND $FECL_3$

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Abstract

Hydrogen peroxide (H2O2) and iron can be toxic to human cells and produce highly reactive oxygen species (ROS) such as hydroxyl radicals (OH•). These ROS can damage cellular components like DNA, proteins, and lipids, leading to cell injury or death. Olive oil is one of the most important components of the Mediterranean diet characterized by many therapeutic proprieties and health protective effects, mainly attributed to its high content of monounsaturated oleic acid, vitamin and polyphenolic compounds. The aim of this study was to evaluate the *in vitro* antioxidant activity of the phenolic extracts of two Algerian olive oil varieties (Chemlal and Sigoise) and to determinate their protective effect against lipid peroxidation. We have used Folin-Ciocalteu reagent method to estimate the phenolic content of the studied extracts. The antioxidant activity was determined using three different antioxidant assays (DPPH scavenging activity, reducing power and hydrogen peroxide **s**cavenging assay). The protective effect of olive oil extract against lipid peroxidation was evaluated in an *in vitro* model of rat liver homogenates through the production of TBARS. The quantitative analysis results showed that the phenolic extracts of Algerian olive oil are rich in phenolic compounds mostly flavonoids, with the highest content found in Chemlal oil (366.095± 1.01 mg GAE/Kg for polyphenols and 79.065± 0.84 mg QE/Kg for flavonoids). Moreover, the studied extracts showed very important anti-free radical activity and reducing power, wherein Chemlal oil exhibited the highest antioxidant activity. The tested extracts demonstrated a strong protective effect against lipid peroxidation, with Chemlal olive oil showing the highest protective effect. These findings confirmed the medicinal use of Algerian olive oils as natural agents in food and pharmaceutical industries

Keywords: olive oil, polyphenols, oxidative stress, biological activities.

1. Introduction

Hydrogen peroxide (H_2O_2) is a ubiquitous reactive oxygen species (ROS) produced by various cell types as a byproduct of normal cellular metabolism. It plays a dual role in cellular processes, acting as a signaling molecule at low concentrations and as a cytotoxic agent at high concentrations. Hydrogen peroxide produces hydroxyl radicals that induce lipid peroxidation in exposed cells, which can lead to DNA damage and cell death (Finkel and Holbrook 2000). Iron is an essential mineral required for various biological processes in the human body, including oxygen transport, energy production and DNA synthesis. However, Iron is toxic to cells in excessive amounts. It can be involved in the generation of harmful ROS through the Fenton reaction and increases lipid peroxidation, leading to membrane damage to mitochondria, microsomes, and other cellular organelles (Goyer, 1996). The toxicity of H2O2 and iron depends on various factors, including concentration, exposure time, and the cell type involved. Free radicals and oxidative stress are recognized as important factors responsible for many chronic diseases and have remained a major health problem. Nutritional compounds with antioxidant activity have received particular attention due to their ability to reduce oxidative stress. The Mediterranean diet is characterized by its health promoting properties, which are attributed to high consumption of vegetables and fruits, low consumption of red meat, and high consumption of olive oil (Loizzo et al., 2012). The beneficial properties and health-protecting effects of olive oil are attributed to its chemical composition, with high content of triglycerides, vitamin, and fatty acids, wherein the monounsaturated omega-9 fatty acid and oleic acid always being the main components (55–83%) (Rossi et al., 2017). Recently, olive oil's phenolic fraction has received much attention due to its health benefits (Laincer et al., 2014). Phenolic compounds contributing to the organoleptic properties of olive oil, act as radical scavengers; they are mainly responsible for the defense against autoxidation of unsaturated fatty acids. In recent years, scientists have also focused their interest on the preventive effect(s) of polyphenols against reactive oxygen species (ROS) mediated degenerative diseases (Manna et al., 2002). According to the literature, the antioxidant properties and the protective effect against lipid peroxidation of Algerian olive oil phenolic extract (OOPE) have been little studied. Therefore, the aim of this study was to evaluate the *in vitro* antioxidant activity of the phenolic fraction of two varieties of Algerian olive oil (Chemlal and Sigoise) and determinate their protective effect against lipid peroxidation.

2. Materials and Methods

2.1. Biological Material

The two varieties of olive oils (Chemlal and Sigoise) used during this study were purchased from two different regions of Algeria; Chemlal variety was purchased from the region of Tizi Ouzou (Latitude: 36°42′42″ North Longitude: 4°02′45″ Est) and Sigoise variety from the region of Mascara (Latitude: 35°31′41″ North Longitude: 0°11′37″ East).

2.2. Extraction of Phenolic Compounds

The phenolic compounds of the tested olive oils were extracted by mixing 5 g of oil with 2 ml of hexane for better oil fluidification. 5 ml of ethanol (70%) was subsequently added. The Mixture was vortexed for 10 min. The emulsion was centrifuged for 20 min at 5500 g at 4 C° to separate the two phases. The alcoholic extract was recovered and this procedure was repeated three times. Finally, the alcoholic extract was evaporated in cold and reduced pressure conditions. The dried extract was resuspended in 1 ml of ethanol (70%) (Nigro et al., 2019).

2.3. Determination of the Antioxidant Activity of the Studied Extracts

2.3.1. Ferric Reducing Antioxidant Power (FRAP) Test

The method of Yen and Duh (1993) with slight modifications was used to evaluate the ferric reducing antioxidant power (FRAP) of the studied extracts. 2.5 ml of each extract solution at various concentrations (31.25 to 500 µg/ml) was mixed with 2.5 mL of potassium ferricyanide (1%) and phosphate buffer (2.5 mL, 0.2 M, pH 6.6). The mixtures were incubated for 20 min at 50°C. After incubation, 2.5 mL of Trichloroacetic acid (10%) was added to the mixtures, which was then centrifuged at 3000 rpm for 10 min. 1 mL of the upper layer was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride (0.1%). Ascorbic acid and gallic acid were used as reference standards. The increase in absorbance provided an indication of higher reducing powers of the samples being analyzed. The reducing power of each extract sample and standard was expressed as the value of the effective 50% concentration (EC_{50}) that correspond to the sample's concentration required for an absorbance equal to 0.5 at 700 nm. The lowest EC_{50} corresponds to the most important activity.

2.3.2. Free Radical Scavenging Activity (DPPH) Test

The antioxidant scavenging activity was studied using 1,1-diphenyl- 2-picrylhydrazyl free radical (DPPH) test as described by Tien et al. (2005) with some modifications. 1.5 mL of each solution at various concentrations (1 to 0.015 mg/ml) was mixed with 1.5 mL of a 0.2 mM ethanolic DPPH solution. After an incubation period of 30 min at 25 °C, the absorbance at 517 nm, the maximum absorbance wavelength of DPPH, was recorded as a (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as a (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the equation:

$$\% \textbf{ inhibition} = \textbf{100} \times \frac{\textbf{A (blank)} - \textbf{A (sample)}}{\textbf{A (blank)}}$$

The antioxidant activity of each phenolic extract was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid and Gallic acid were used as standards. All measurements were performed in triplicate.

2.3.3. Test of Hydrogen Peroxide Scavenging Capacity

The ability of the phenolic extracts to scavenge hydrogen peroxide was determined according to the method of Kumar et al. (2007). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (0.1 M, pH7.4). 2 ml of each solution extract at different concentrations (0.5 mg/mL to 0.06 mg/mL) in ethanol (70%) was added to a hydrogen peroxide solution (1.2 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined, 10 min later, against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of phenolic extract and standard compounds were calculated as follows:

$$\% \textbf{ Scavenged } [\mathbf{H_2O_2}] = \mathbf{100} \times \frac{\mathbf{AC - AS}}{\mathbf{AC}}$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the phenolic extracts or standards. Hydrogen peroxide scavenging capacity of the studied phenolic extract was expressed as IC_{50}, defined as the concentration of each tested extract required to cause a 50% decrease in the initial H_2O_2 concentration. Ascorbic acid and Gallic acid were used as standards. All measurements were performed in triplicate.

2.4. Evaluation of the Protective Properties of Olive Oil s against Lipid Peroxidation

2.4.1. Preparation of Liver Homogenate

The liver was isolated from 3 normal albino Wistar rats. The homogenate 10% (w/v) was prepared in phosphate buffer (0.1 M, pH 7.4 having 0.15 M KCl) using the homogenizer at 4°C. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant obtained was used for this study (Kuma et al., 2013).

2.4.2. In vitro Evaluation of the Protective Effect against Lipid Peroxidation

Lipid peroxidation is an oxidative breakdown of polyunsaturated fatty acids in cell membranes, producing malondialdehyde (MDA) and 4-hydroxynonenal and causing cell damage. Measuring malonaldehyde (MDA) is an indicator of lipid peroxidation (Ait Abderrahima et al., 2019). The method described by Gupta and Sharma (2010) was used to evaluate the protective effect of the tested extracts against lipid peroxidation. 580 µL of Phosphate buffer (0.1 M; PH 7.4) was mixed with 200 µL of extract or standard and 200 µL liver homogenate. 20 µL of ferric chloride (100 mM, H_2O_2 0.50% prepared in phosphate buffer 0.1 M, pH 7.4) was then added to the mixture that was placed in a shaking water bath for 1 h at 37°C. The assessment of malonic dialdehyde (MDA) content was determined according to the method described by Yagi (1976). A volume of 800 µL of TBA (0.375% w/v) was added to 200 µL of the previously prepared solution. After shaking for 2 min, the mixture was incubated in a water bath at 100°C for 10 min. During this step, the aldehyde functions of MDA were released by acid hydrolysis at 100°C. They react with TBA forming a pink colored complex (MDA-TBA). To stop this reaction, the tubes were placed

in ice and the complex thus formed was extracted with 2 ml of 1-butanol for 2 min. After centrifugation at 4000 rpm for 10 min at 4°C (Sigma, 3K10, Laborzentrifugen, Germany), the supernatant was collected and the absorbance of the pink chromogen obtained was measured at 532 nm using spectrophotometer (Shimadzu 1240, Japan). The tissue concentration of malondialdehyde (MDA) was calculated using a linear PET curve. The percentage of MDA inhibition was determined according to the formula:

$$\mathbf{MDA}\ (\%)\ = \mathbf{100x}\frac{\mathbf{C0} - \mathbf{C1}}{\mathbf{C0}}$$

Where C_0 is the MDA concentration without protection and C_1 is the MDA concentration with protection.

Statistical analysis

All assays were performed in triplicate and the results represent the means ± standard deviation. The data analysis was performed using the Statistica StatSoft software (version 6.1, Statsoft, Tulsa, UK). The one-factor ANOVA was used to compare the means, followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

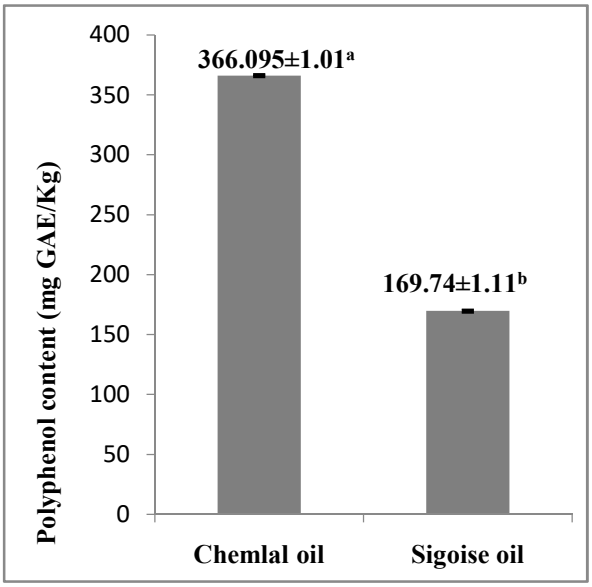
3. Results and Discussion

3.1. Antioxidant Activity

3.1.1. Phenolic and Flavonoid Content

Polyphenols are one of the most important bioactive compounds found in olive oil. They are responsible for its organoleptic properties (aroma and flavor). They are mainly characterized by their antioxidant activity and olive oil protection from oxidation. The total polyphenol and

flavonoid content of the studied extracts is illustrated in Figure 1 and Figure 2.



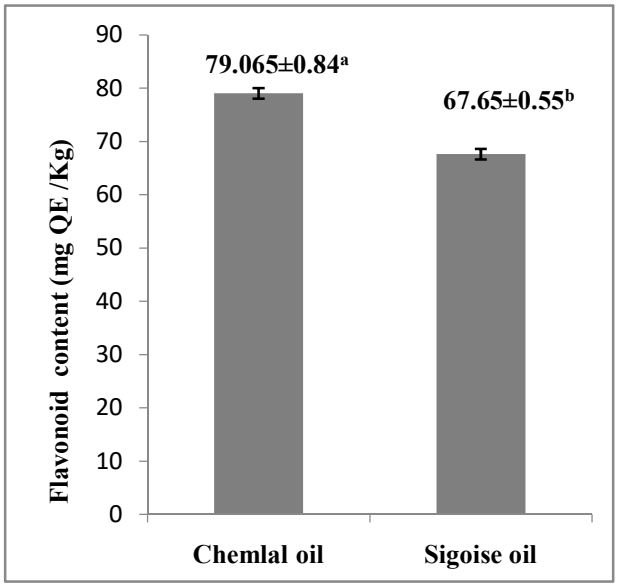


Fig. 1. Polyphenol content of extracts **Fig. 2. Flavonoid content of extracts**

(The values followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$).

3.1.1. 1. Polyphenol Content

The results of the present study indicated that olive oil extracts are rich in phenolic compounds. Chemlal olive oil presented the highest polyphenol content (366.095± 1.01 mg GAE/Kg) compared to Sigoise olive oil (169, 74±1.11 mg GAE/Kg). The total polyphenol content of the tested Algerian olive oils was higher than those reported by Bouchnak *et al*. (2018) (107 ± 1,317mg EAG/ kg), and lower than those previously reported for other olive oil samples from Spain (Bekkouche et al., 2013), Greece (Kalogeropoulos et al., 2014), and Tunisia (Loubiri et al., 2016). The concentration and the type of phenolic compounds in olive oil vary according to the olive variety climate, the condition and method of extraction, storage and maturation conditions, and commercial storage of the final product (Nigro et al., 2019).

3.1.1. 2. Flavonoids Content

Flavonoids are low atomic weight phenolic compounds crucial for the smell and which at the same time have antioxidant properties. In the present study, Chemlal olive oil showed higher flavonoid content (79.065± 0.84 mg QE/Kg) compared to Sigoise olive oil (67.65±0.55 QE/Kg). The variation in the flavonoid content of olive extracts depends on the olive variety climatic and environmental conditions.

3.1.2. Ferric Reducing Antioxidant Power

The results of the reducing power of the standers and the extracts are depicted in Figure 3.

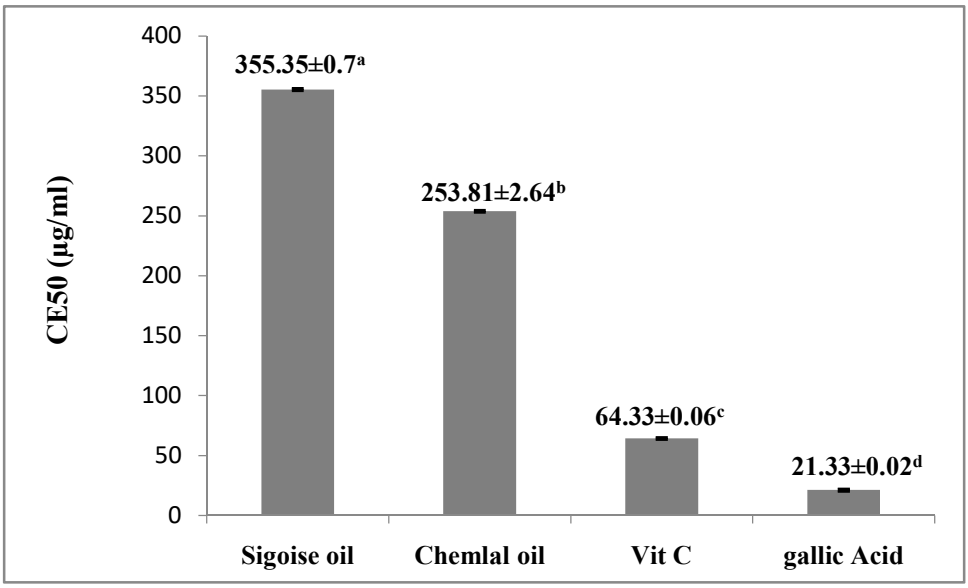


Fig. 3. Reducing power of standards and extracts .

(The values followed with different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$).

The reducing power determines the capacity of an antioxidant to donate an electron**.** The antioxidant compounds trap the fee radicals and prevent the peroxidation chain reaction. The antioxidant activity of the tested olive oils evaluated by the reducing potential test revealed that Chemlal and Sigoise oils have important reducing powers with EC_{50} values of 253.81±2.64 µg /mL and 355.35±0.7µg /mL respectively. These reducing powers are much lower than those of gallic acid (EC_{50} = 21.33±0.02µg/mL) and vitamin C (EC_{50}= 64.33±0.06 µg/mL). The reducing power of Chemlal oil was significaly higher than that of Sigoise variety, possibly because of Chemlal's higher phenolic content. The classification of the reducing power in the decreasing order of strength is as follows: Gallic acid > Ascorbic acid > Chemlal olive oil > Sigoise olive oil. We found a positive correlation between the total polyphenol content and the reducing power for the two studied extracts with a high coefficient of correlation (r) for both Chemlal (r = 0.998) and Sigoise (r =0.997) olive oil varieties. Our finding are similar to the results of other scientific research conducted by Loizzo et al., (2012) and Song and Barlow, (2004) who found a strong correlation between the phenolic content and FRAP assay. Manna et al. (2002) evaluated the antioxidant effect of the methanolic extracts of six virgin olive oils by the FRAP test. Loizzo et al. (2012) found that phenolic extract of virgin olive oils have an antioxidant activity evaluated by the reducing power (FRAP) test. It is evident that the antioxidant activity of olive oil extracts is attributed to the redox property of their phenolic compounds, which acts as a donor of electrons or hydrogen atoms, allowing free radical scavenging or the decomposition of peroxides, there by quenching a single and triplet oxygen (Loizzo et al., 2012).

3.1.3. DPPH Radical Scavenging Activity

The DPPH test determines the ability of an antioxidant compound to act as a donor of hydrogen atoms. The results of the DPPH free radical scavenging activity of the tested extracts are shown in Figure 4.

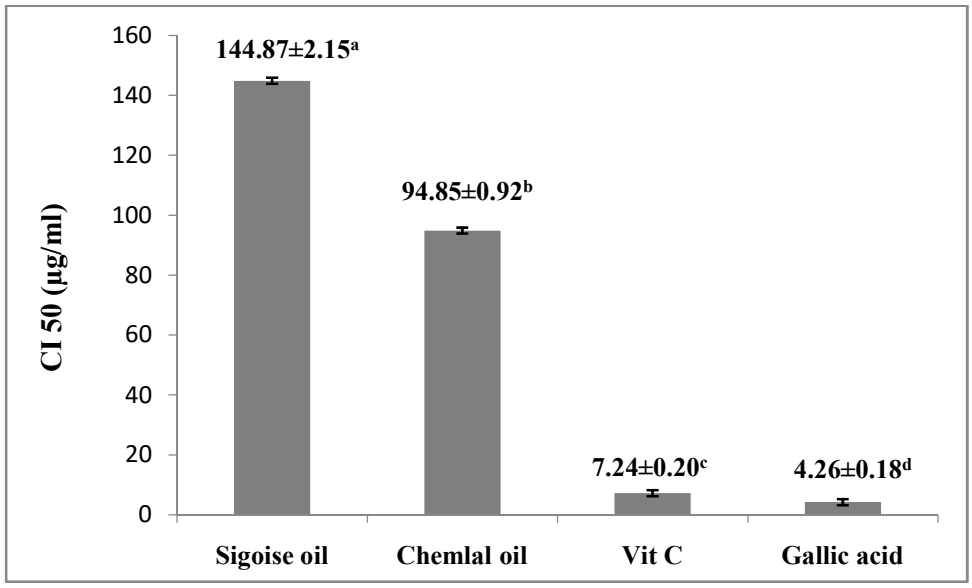


Fig.4. Results of DPPH test of standards and extracts.(The values followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$).

The results of the DPPH tests showed that the studied olive oils have an important antiradical activity with IC_{50} values of 94.85±0.92 µg/mL for Chemlal oil and 144.87±2.15 µg/ mL for Sigoise oil. This activity is lower than that of the standard antioxidants (ascorbic acid and gallic acid), which have an important antiradical activity with IC_{50} values of 7.24± 0.20µg/mL and 4.26±0.18 µg/mL, respectively. As for the reducing power, it clearly appears that the antiradical activity of Chemlal olive oil is higher than that of Sigoise variety. This is may be attributed to its high flavonoids and polypheol content compared to Sigoise oil. Therefore the antiradical activity was classified in the following descending order: Gallic acid > Ascorbic acid > Chemlal olive oil > Sigoise olive oil. A strong correlation was noted between the phenolic content and the DPPH radical scavenging activity with high coefficients of correlation (r=0.964 for Chemlal oil and r =0.996 for Sigoise oil). These result are similar to those reported by Laincer *et al*. (2014) for Algerian cultivars (r=0.76). The result of a study by Loizzo et al. (2012) indicated that the phenolic extracts of extra virgin olive oils demonstrated a strong radical scavenging activity with IC_{50} ranging from 56.5±1.8 to 106±1.8µg/ml. Bouchenak et al. (2018) reported that five Algerian virgin olive oils have antiradical activity and reduce the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Negro et al. (2019) demonstrated that the Italian mono varietal extra

virgin olive have an antioxidant activity evaluated by DPPH test with IC_{50} values ranging from 91 to160 mg/ml of oil.

3.1.4. Hydrogen Peroxide Scavenging Activity

Recently, several methods to measure the antioxidant capacity of natural products and plant extracts have been developed. However, few studies have evaluated their hydrogen peroxide scavenging activity. The results of hydrogen peroxid scavenging capacity of the studied extracts and standard antioxidants (vitamin C and gallic acid) are illustrated in Figure 5.

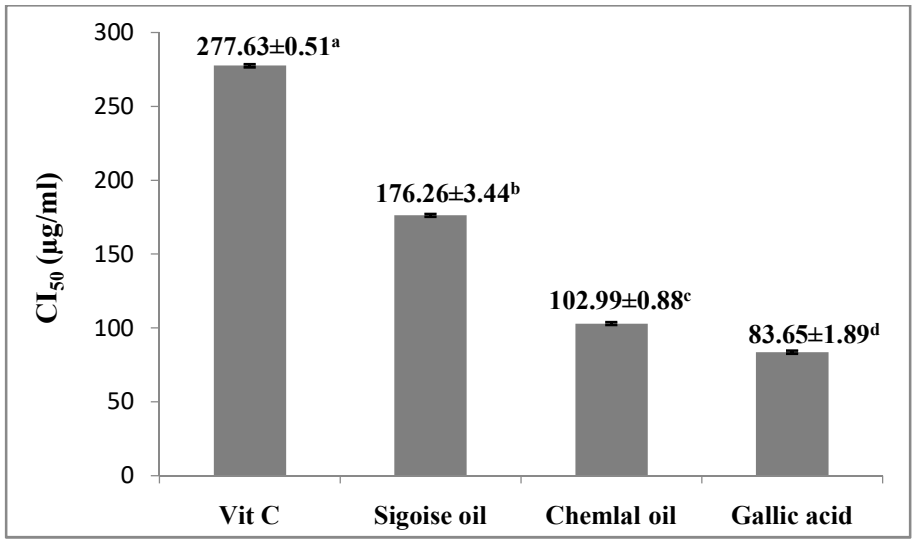


Fig. 5. Hydrogen peroxide scavenging activity (%) of standards and extracts.

(The values followed with different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$).

Hydrogen peroxide can be generated by biological systems through many oxidizing enzymes such as superoxide dismutase. H_2O_2 is known to be toxic and induces cell death in vitro; it can attack many cellular energy producing systems. It acts as a toxicant to the cell by converting to hydroxyl radicals in the presence of metal ions and superoxide anions, and produces singlet oxygen by reacting with superoxide anion (MacDonald-Wicks et al., 2006). Therefore, H_2O_2 removal is very important for antioxidant defenses in cell or food systems. Our results indicated that the studied olive oil have an important hydrogen peroxide scavenging capacity. In addition, Chemlal olive oil showed a better scavenger activity (CI_{50}=102.99±0.88µg/ml) then Sigoise olive oil (IC_{50}=176.26±3.44µg/ml). The studied extracts have higher scavenging capacity than that of ascorbic acid, which showed the least scavenging activity with an IC_{50} of 250.48±0.74µg/ml. Similar results were found with other natural plants extracts such as ginger and clove essential oils (Bellik et al., 2010 and Abdellah et al., 2021). Gallic acid showed the highest scavenging capacity of hydrogen peroxide compared to the studied compounds, with an IC_{50} value of 83.65±1.89 µg/mL. The classification of the scavenging activity in the decreasing order of

strength is as follows: Gallic acid > Chemlal olive oil > Sigoise olive oil > Ascorbic acid. The scavenging activity of olive oils was affected by the phenolic content with a strong coefficient of correlation (r= 0.992 for Chemlal oil and r = 0.995 for Sigoise oil). The antioxidant capacity of olive oil could be attributed to the presence of some bioactive compounds such as oleuropein, hydroxyterisol and tyrosol, which are characterized by strong antioxidant and radical scavenging activities (Tuck et al., 2002).

3.2. Protective Effect of Olive Oil Extracts against Lipid Peroxidation

The results of the protective effect of olive oil extracts and standards against lipid peroxidation are illustrated in Figure 6.

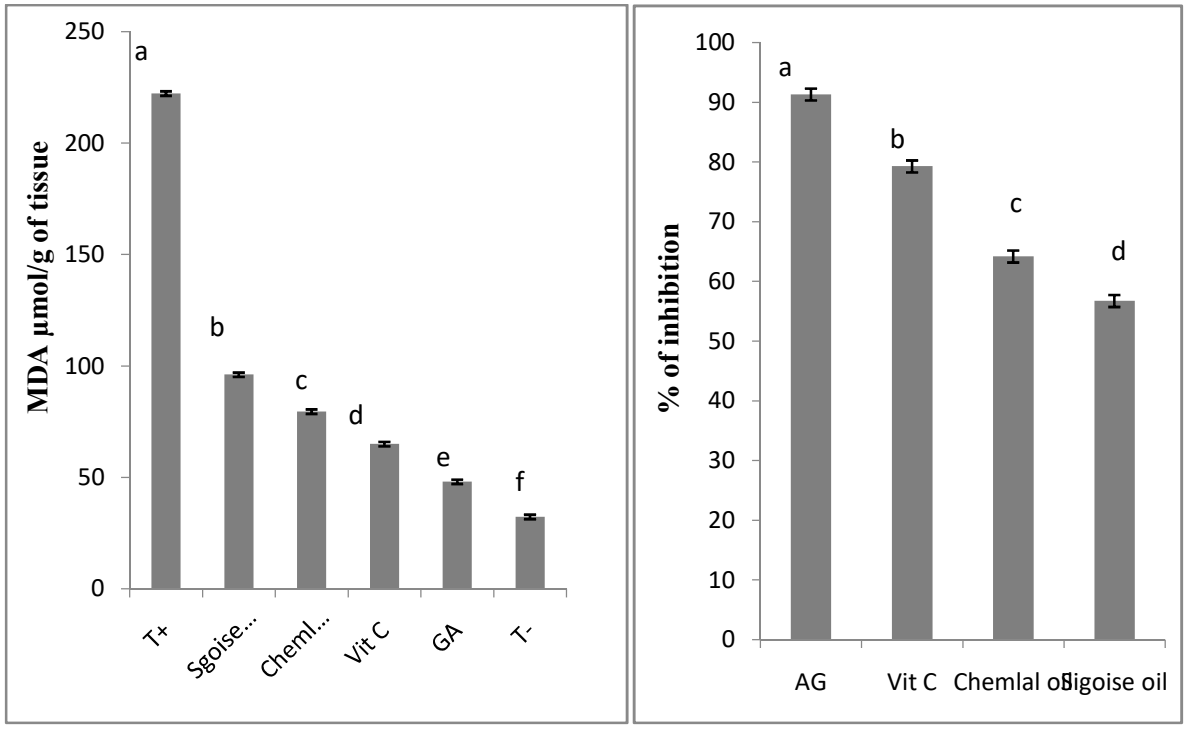


Fig. 6. Protective effect of olive oil extracts, gallic acid and ascorbic acid against lipid peroxidation expressed in (A) MDA µmol/g of tissue, and (B) the percentage of peroxidation inhibition. (The values followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$).

The obtained results indicated that olive oil extracts possess a protective effect against lipid peroxidation when compared with the positive control that showed a higher concentration of MDA (222.12 ± 0.88 µmol/g) than the normal (negative) control (32.4±0.58 µmol/g). The extract of Chemlal olive oil was the most effective against lipid peroxidation with the lowest MDA content (79.6±0.46 µmol/g) and the best inhibitory effect (64.19 ±0.21%) compared to the extract of Sigoise olive oil, which had an inhibition of 56.74 ±0.21% and an MDA content of 96.15±0.47 µmol/g . Gallic acid and vitamin C used as controls significantly ($p < 0.05$) inhibited

lipid peroxidation as demonstrated by the level of MDA (48.14± 1.87 µmol/g for gallic acid and 65.03±0.46 µmol/g for vitamin C) and the higher inhibitory potential (91.32 ± 0.33% for gallic acid C and 79.29±0.% for vitamin), as compared to the extract samples. This protective activity of olive oil extracts may be attributed to the effect of bioactive compounds found in olive oil. According to the literature, oleuropein is one of the most abundant compounds in olive product; it prevents heart disease and protects cell membrane from lipid oxidation (Nicoli et al., 2019). Manna et al. (1999) reported that hydroxytyrosol, one of the most important polyphenols in olive oil, protected human erythrocytes against lipid peroxidation induced by H_2O_2. In vivo study recorded that olive leaf extracts are rich in phenolic compounds such as oleuropein and hydroxytyrosol and reduced TBARS values in Wistar rat livers (Paiva-martins et al., 2010). It was also found that the polyphenol of olive oil protect blood lipid against oxidative stress (Manna et al., 2002). Our results suggest that the olive oil extract may efficiently prevent the initiation of lipid peroxidation and protect human body against oxidative stress. This finding confirmed the health benefit of olive oil extensively used in Mediterranean diet.

4. Conclusion

The results of our study confirmed the beneficial health proprieties of the Algerian olive oil and showed that their phenolic extracts are rich in bioactive compounds exhibiting high protective effects against lipid peroxidation due to their important antioxidant activity. Their Hydrogen peroxide scavenging activity was especially stronger than that of vitamin C. This allows envisaging their use as a potential source of natural antioxidants which can be used in the food and pharmaceutical industry as protective compounds against oxidative stress and lipid peroxidation. Further in vivo studies would clarify the protective effects of the phenolic compounds of the Algerian olive oil. The isolation, characterization, purification and bioavailability of these compounds are needed for the therapy of diseases caused by oxidative stress such as cardiovascular disorders and cancer.

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Declaration of interests

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this article.

References

Abdellah, F., Boubakeur, B., Ayad, N., Boukraa, L., Hammoudi, S.M., Boumedienne, A., Benaraba, R., 2021.Chemical composition and biological activities of *Eugenia caryophyllata* thunb essential oil. Asian J App Chem Res. 9(4), 51-62. http://dx.doi.org/10.9734/ajacr/2021/v9i430224.

Ait Abderrahim, L., Taïbi, K., Ait Abderrahim, N., Alomery, A.M., Abdellah, F., Alhazmi, A.S., Aljassabi, S., 2019a. Protective effects of melatonin and N-acetyl cysteine against oxidative stress induced by microcystin-LR on cardiac muscle tissue. Toxicon 169, 38–44. https://doi.org/10.1016/j.toxicon.2019.08.005.

Bakhouche A, Lozano-Sanchez J, Beltran-Debon R, Joven J, Segura Carretero A and Fernandez-Gutierrez A.2013. Phenolic characterization and geographical classification of commercial Arbequina extra-virgin olive oils produced in southern Catalonia. Food Res. Int. 50, 401–408. http://dx.doi.org /10.1016/j.foodres.2012.11.001.

Bellik ,Y., Benabdeslselam, F., Ayad, A., Dahmani, Z., Boukraa, L., Nemmar, A., Iguer-Ouda, M., 2013.Antioxidant activity of the essential oil and oleoresin of *Zingiber officinale* Roscoe as affected by chemical environment. Inter J food prop.16, 1304-1313. . http://dx.doi.org/10.1080/10942912.2011.584257.

Finkel, T., and Holbrook, N.J., 2000. Oxidants, Oxidative Stress and the Biology of Aging. Nature, 408, 239-247. http://dx.doi.org/10.1038/35041687.

Goyer, R.A., 1996. Toxic effects of metals. In: Klaassen CD, ed. Casarett & Doull's toxicology: the basic science of poisons. 5th ed. New York City, NY: McGraw-Hill.1996,715-716.
Gupta, V., Sharma. M., 2010. Protective effect of *cinnamomum tejpata* on lipid peroxide formation in isolated rat liver homogenate. Curr Res J of Biol Sci. 2(4), 246-249.

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., Sayadi, S., 2008. Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chem Biol Interact. 176, 88–98. https://doi.org/ 10.1016/j.cbi.2008.08.014.

Kalogeropoulos, N., Tsimidou, MZ., 2014. Antioxidants in greek virgin olive oils. Antioxidants.3,387–413. https://doi.org/10.3390/antiox3020387.

Kumar, S., Kumar, D., Singh, N., Vasiasht, B.D., 2007. *In vitro* free radicals scavenging and antioxidant activity of *Moringa oleifera* pods. J Herb Med Toxicol.1, 17-22. . https://doi.org /10.1016/j.jams.2012.03.002.

Kumar, S., Mishra, A., Pandey, A.K., 2013. Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models. Complement Altern. Med . 13,120. https://doi.org/10.1186/1472-6882-13-120.

Laincer, F., Laribi, R., Tamendjari, A., Arrar, L., Rovellini,P., Venturini, S.,2014.Olive oils from Algeria: Phenolic compounds,antioxidant and antibacterial activities. Grasas y aceites. 65 (1). https://doi.org/10.3989/gya.035713 .

Loizzo, MR., Di Lecce, G., Boselli,E., Menichini, F., Frega, N. G., 2012.Radical Scavenging, Total Antioxidant Capacity,and Antiproliferative Activity of Phenolic Extracts from Extra Virgin Olive Oil by Cultivar 'Frantoio'. Int J Food Prop.15(6), 1345-1357. https://doi.org/10.1080/10942912.2010.522291.

Loubiri, A., Taamalli, A., Talhaoui, N., Mohamed, S.N, Carretero, A., Zarrrouk, M.,2016. Usefulness of phenolic profile in the classification of extra virgin olive oils from autochthonous and introduced cultivars in Tunisia. Eur Food Res Technol. 3,467–479. https://doi.org/10.1007/s00217-016-2760-7.

MacDonald-Wicks, L.K., Wood, L.G., Garg, M.L., 2006. Methodology for the determination of biological antioxidant capacity in vitro: A review. J Agric Food Chem.86, 2046–2056. https://doi.org/10.1002/jsfa.2603.

Manna, C., D'angelo, S., Migliardi, V., Loffredi, E., Mazzoni, O., Morrica, P., Galletti, P., Zappia, V., 2002. Protective effect of the phenolic fraction from virgin olive oils against oxidative stress in human cells. J Agric Food Chem. 50, 6521-6526. https://doi.org/10.1021/jf020565+ .

Manna ,C., Galletti, P., Cucciolla, V., Montedoro, G., Zappia, V., 1999. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. J Nutr Biochem. 10,159–165. https://doi.org/10.1016/s0955-2863(98)00085-0.

Negro, C., Aprile, A., Luvisi, A., Nicolì, F., Nutrica, E., Vergine, M., Miceli, A., Blando, F., Sabella, E., De Bellis, L., 2019. Phenolic Profile and Antioxidant Activity of Italian Monovarietal Extra Virgin Olive Oils. Antioxidants. 8, 161. https://doi.org/10.3390/antiox8060161.

Nicolì, F., Negro, C., Vergine, M., Aprile, A., Nutricati, E., Sabella, E., Miceli, A., Luvisi, A., De Bellis, L., 2019. Evaluation of Phytochemical and Antioxidant Properties of 15 Italian Olea europaea L. Cultivar Leaves.Molecules. 24, 1998. https://doi.org/10.3390/molecules24101998.

Paiva-martins, F., Fernandes, J., Santos, V., Silva, L., Borges, F., Rocha, S., Belo, L., Santos-silva, A., 2010. Powerful protective role of 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde against erythrocyte oxidative-induced hemolysis. J Agric Food Chem.58, 135–140. https://doi.org/10.1021/jf9031052.

Soong, Y.Y., Barlow, P.J., 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemi. 88, 411–417. https://doi.org/10.1016/j.foodchem.2004.02.003.

Tien, Y.Y., Ng, C.C., Chang, C.C., Tseng, W.S., Kotwal, S., Shyu, Y.T.,2005. Studies of the lactic-fermentation of sugar apple (*Annona squamosa L.)* puree. J Food Drug Anal. 13,377-381. https://doi.org/10.38212/2224-6614.2572.

Tuck, K.L., Hayball ,P.J.,2002. Major phenolic compounds in olive oil: Metabolism and health effects. J Nutr Biochem. 13(11),636-644.https://doi.org/10.1016/s0955-2863(02)00229-2.

Yagi, K., 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochemia Medica. 15(2),212-216. https://doi.org/10.1016/0006-2944 (76)90049-1.

Yen, G.C., Duh, P.D., 1993. Antioxidative properties of methanolic extracts from peanut hulls. J Am Oil Chem Soc.70, 383–386. https://doi.org/10.1007/BF02552711.