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Research Article

Polyphenolic Profile and Antioxidant Activities of *Olea Europea* Bark and Root Extracts

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ABSTRACT

In recent decades, there has been a growing interest in the study of medicinal plants and their traditional use for the treatment of various diseases. The olive tree, or *Olea europaea* L., is an inseparable entity of the Mediterranean peoples. This plant belongs to the large family of Oleaceae. The aim of this study was to evaluate the antioxidant activity of aqueous and methanolic extracts of olive roots and bark, respectively (RAE, RME, BAE, and BME). The determination of total polyphenols by the Folin-Ciocalteu method revealed the richness of RME and BME extracts in phenolic compounds; the contents are respectively, 509.33 µgGAE/mg and 387.33 g GAE/mg of extract. The radical scavenging activity towards DPPH showed that BME and RME extracts are the most active, with an IC_{50} of 0.404 mg/mL and 0.525 mg/mL, respectively. In the ß-carotene bleaching assay, the extracts RAE, BME, and BAE exhibited the highest inhibitory activities, with 90.19%, 86.15%, and 78.05%, respectively. The antioxidant activity of RME extract is confirmed on oxidative homolysis of red blood induced by H_2O_2 with a percentage of inhibition (21%). These findings provide evidence that *Olea europea* bark and roots are potential sources of antioxidant which have many benefits towards human health.

INTRODUCTION

Olive tree (*Olea europaea* L.) is one of the most important fruit trees in the Mediterranean countries; they cover 207.822 ha in Algeria [1]. It has significant economic and social significance, and it may be a source of prospective advantages obtained from the use of any of its by-products. Preparations of *Olea europaea* have been widely used in folk medicine in the European Mediterranean region, the Arabian Peninsula, India, and other tropical and subtropical regions as a diuretic, hypotensive, emollient, and for urinary and bladder infections [2].

The possible toxicity of several synthetic substances used in food has increased interest in natural items in recent years. Some industries, such as those involved in the manufacturing of food additives, cosmetics, and medicines, have boosted their efforts to extract and purify bioactive molecules from natural materials. Thus, a need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years [3, 4].

The antioxidant capacity and the oxidative stability were substantially improved by adding olive leaves extract to the commercially available oils, olive, sunflower, and palm oils [5]. The protective effects of diets rich in fruit and vegetables against cardiovascular diseases and some cancers have been attributed partly to the antioxidant compounds contained therein, particularly to the polyphenol compounds [6]. Flavonoids are a widely distributed group of polyphenol compounds and are identified as antioxidants in various biological systems [7]. Researchers have focused on antioxidant compounds derived from the leaves

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and fruit of *Olea europea*, however, not much studies are dealing with the bark and roots of this plant.

The aim of the present study was to investigate the phenolic profile of Olea *europaea* roots and bark extracts and to evaluate their antioxidant activity.

MATERIALS AND METHODS

Plant Material

The bark and the roots of *Olea europea* were collected in March 2022, from Mansoura in eastern Algeria. Bordj BouArréridj. Plant material was cleaned, dried, and ground using an electric micronizer.

Preparation of Extracts

One hundred grams of the powdered plant material were soaked in methanol or water (1/10, w/v) for 24 h with frequent agitation. After filtration and solvent evaporation in a rotary evaporator, the obtained extracts were stored in darkness at 4°C until use. The following formula was used to calculate the extraction yield of each sample:

$$\%\ \text{yield of each sample} = \frac{\text{weight dried extracts g}}{\text{weight dry plant g}} \times 100$$

Determination of Total Phenolic Content

Phenolic content was assessed by Folin-Ciocalteu reagent as described by Li et al. [8]. Two hundred micro litres of diluted sample were added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 µL of saturated sodium carbonate (75 g/L) was added. The absorbance at 765 nm was measured after 2 hours of incubation at room temperature. The standard calibration curve was made with gallic acid (0-160 g/mL). The results were expressed as Gallic acid equivalent (GAE)/g extracts.

Determination of Flavonoid Content

Aluminum-chloride colorimetric assay was used to determine the total flavonoid content [9].One mL of extracts was mixed with the same volume of 2% aluminium trichloride (AlCl3) solution. after 10 min of incubation, the absorbance of the mixture was measured at 430 nm. Quercetin was used for the standard calibration curve. The data were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract).

DPPH Free Radical Scavenging Assay

The radical scavenging activity of the studied extracts against the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was evaluated as described by Meziti et al. [10]. 2.5 mg of DPPH was dissolved in 100 mL of methanol to make the DPPH solution., 25µL of extract or standard antioxidant was added to 975µL of DPPH solution. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature and the decreases in the absorbance values were measured at 517 nm. The following formula was used to compute the percentage of DPPH scavenging activity.

$$\%\text{DPPH scavenging activity} = \left(\frac{\text{A control} - \text{A sample}}{\text{A control}}\right) \text{x } 100$$

Where A sample is the absorbance of the test compounds, and A control is the absorbance of the control reaction mixture without the test compounds. The IC_{50} values were used to reflect the concentration of extract that resulted in 50% neutralization of DPPH radicals, it was estimated using the plot of inhibitory percentages against concentration.

β-Carotene Bleaching Assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation according to the method of Kartal et al. [11].

β -carotene/linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL of chloroform, and 25µL linoleic acid and 200 mg Tween 40 were added. After evaporation of chloroform, 100 mL distilled water, saturated with oxygen (30 min 100 mL/min), were added with vigorous shaking; 2.5 mL of this reaction mixture were spread into test tubes and 350 µL of the extracts prepared at 2 mg/mL concentrations, were added, the emulsion was incubated for 48 h at room temperature and the absorbance (490 nm) was recorded at different time intervals. The same procedure was repeated with the synthetic antioxidant vitamin C, and a blank or negative control (containing only methanol).

The inhibition percentage of β-carotene bleaching was calculated according to the following formula:

$$\%\ inhibition = \left(\frac{A\,T48h - AC48h}{A\,C0h - AC48h}\right) \times 100$$

Where, A T48h and A C48h were the absorbance values measured after incubation for 48 hours for test sample and control, respectively. A C0h Absorbance of negative control at zero time of the incubation.

Inhibition of Erythrocyte Hemolysis

The antihemolytic effect of the studied extract was investigated according to the method of Okoko and Ere [12], After gaining informed agreement, veinous blood was collected from a healthy volunteer and placed into heparinized tubes. Whole blood was centrifuged at 4000 rpm for 10 minutes, then washed three times with phosphate buffered saline (0.2 M, pH 7.4) then resuspended in the same buffer to the appropriate haematocrit level (2%). To induce hemolysis, a portion of erythrocyte (200 µL) was put into a test tube, followed by 100 µL of hydrogen peroxide (3mM and 6mM). The test sample (200 µL) was then added, and the total contents were gently stirred and incubated at 37°C for 3 hours. Phosphate buffered saline (8 mL) was added, and the solution was centrifuged at 3 000 rpm for 10 minutes. Absorbance of the contents was measured at 540 nm. Increase in absorbance indicates greater hemolysis.

Statistical Analysis

The results are given as mean ±SD. A one-way analysis of variance was used to determine the statistical significance of the difference, which was then followed by the appropriate Dunnetts test or Students t-test. At a p-value of <0.05, differences were deemed significant.

RESULTS AND DISCUSSION

Extraction Yield

Table 1 report the extraction yield of bark and roots obtained using two different solvents, water, and methanol. Results showed different amounts of extractable soluble compounds, the yield obtained with bark (9.13% for aqueous extract and 8.86 for methanolic extract, was higher than the yield of roots (8.53%, 7.46% for methanolic and aqueous extract, respectively).

These results are partially consistent with previously published studies, in which the highest extract yield was obtained from bark rather than from wood [13-15], according to Faraone et al. [16]. This result can be explained by the fact that the bark is more subjected to biotic and abiotic stress, causing plants to defend themselves and producing a large amount of specialized metabolites.

Table 1: Yield of bark and roots extracts

Extract	Yield (%)
RME	8.53
RAE	7.46
BME	8.86
BAE	9.13

RME: Roots methanolic extract, RAE: Roots aqueous extract, BME: Bark methanolic extract, BAE: Bark aqueous extract.

Total Phenolic and Flavonoid Contents

Studies on the biological functions of phenolic compounds and flavonoids, which may act as antioxidants, have received a lot of attention [17]. The obtained results in our study showed that roots methanolic extract contained the highest amount of total phenolic (509.33±199.87 µg EAG/mg of extract) followed by bark methanolic extract (387.33±48.08 µg EAG/mg of extract), bark aqueous extract contains (210±27.81 µg EAG/mg of extract). While roots aqueous extract contains only (167.5±6.36 µg EAG/mg of extract).

Table 2: Phenolics and flavonoids contents of *Olea europea* bark and root extracts.

Extract	Polyphenols (µg GAE/mg of extract)	Flavonoids (µg QE/mg of extract)
RME	509.33±199.87	2.97±1.87
RAE	167.5±6.36	8.40±1.90
BME	387.33±48.08	7.41±1.04
BAE	210±27.81	7.92±0.93

RME: Roots methanolic extract, RAE: Roots aqueous extract, BME: Bark methanolic extract, BAE: Bark aqueous extract. The results are given as means ± SD of three measurements.

There are very few studies in the literature that focused on the phenolic content of Olea europea roots and bark. Faraone and his team [16] showed that total phenolic contents of hydromethanolic extract of wood and bark of Olea europea ranged from (130 mg EAG/g to et 125 mg EAG/g) respectively which were much lower than the present results. These differences could be related to the divergence in the repartition of phenolic compounds between the different parts of the plant, different methods of extraction, or different climatic conditions [18-20].

DPPH Radical Scavenging Activity

The ability of antioxidants to scavenge DPPH free radicals is widely recognized and is thus frequently used as a trustworthy test to assess the free radical scavenging capability of various plant extracts. Surprisingly, even at low doses, the DPPH radical test is extremely sensitive to active substances. Another advantage is that this test saves time and may be used to analyze a batch of samples in less time. This method is frequently used to evaluate the free radical scavenging capacity of plant-based antioxidant extracts [21].

The DPPH radical scavenging activity of olive bark and root extracts was tested and compared with ascorbic acid (Fig. 1). The scavenging activity of Ascorbic acid was found to be higher than those of the four extracts. Methanolic extract of bark and roots were more active with IC_{50} 0.404±0.02 mg/mL) et (0.525±0.03 mg/mL) respectively, aqueous roots and bark extracts were less active with IC_{50} (1.786±0.09 mg/mL) and (1.102±0.02 mg/mL) respectively.

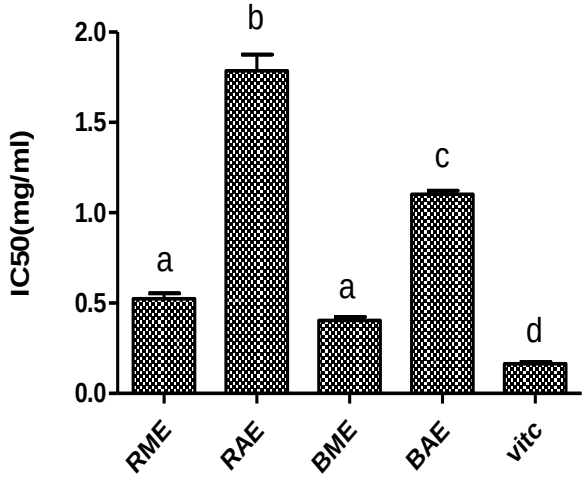

Figure 1: DPPHradical scavenging activities of *Olea europea* bark and root extracts.
RME: Roots methanolic extract, RAE: Roots aqueous extract, BME: Bark methanolic extract, BAE: Bark aqueous extract, vit c: vitamine C. (Values are the average of three measurements ± SD), bars with different letters indicate activities significantly different ($p \leq 0.01$).

Addab and his team [22] tested the effect of leaves ethanolic extract harvested from different regions of eastern Algeriain DPPH assay, the reported IC_{50} values ranged from 84 to 102 mg/mL. Lins and co-workers [23] studied the scavenging activity of methanolic extract prepared from leaves towards DPPH radical, they reported a very low IC_{50} value of 13.8 mg/mL. Nevertheless, direct quantitative comparisons cannot be made due to differences in the experimental conditions.

The radical scavenging activity of *Olea europea extracts* is probably attributed to the presence of phenolic compounds. It is generally predicted that DPPH radical scavenging activity, and with-it antioxidant activity, is strongly affected by the amount of phenolic compounds as well as the degree of hydroxylation of the phenolic compounds [24]. Indeed, the obtained results in our study indicate the existence of significant linear correlation (r^2=0.75, P<0.05) between IC_{50} values of radical-scavenging activity and total phenolic content. Phenolic compounds are recognized as potentially antioxidant substances with the ability to scavenge free radical species, and reactive forms of compounds are attributed to their low potential redox, which makes them thermodynamically capable of reducing free radicals by a transfer of hydrogen or electron from hydroxyl groups and delocalization of an unpaired electron, resulting in the formation of a stable phenoxyl radical [25].

β-Carotene Bleaching Assay(BCB)

In this test, linoleic acid oxidation generates hydroperoxide-derived free radicals that target the chromophore of b-carotene, bleaching the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of α-carotene may be described as a free radical scavenger and primary antioxidant [26].

As can be seen in Fig. 2 and 3 all the extracts inhibit significantly the β carotene bleaching ($p \leq 0,01$) in comparison with the control which represents 100% of peroxidation. the extracts RAE, BME and BAE showed the highest antioxidant activity with (90.19±3.68%), (86.15±4.49%) et (78.05±0.5%) respectively, these activities are significantly stronger than the activity of RME (37.40±3.06%) which showed the highest radical scavenging activity, these data appear to contrast with those obtained from DPPH and Folin assays, especially for RME which showed the highest antioxidant activity in DPPH assay and the lowest antioxidant activity in BCB.

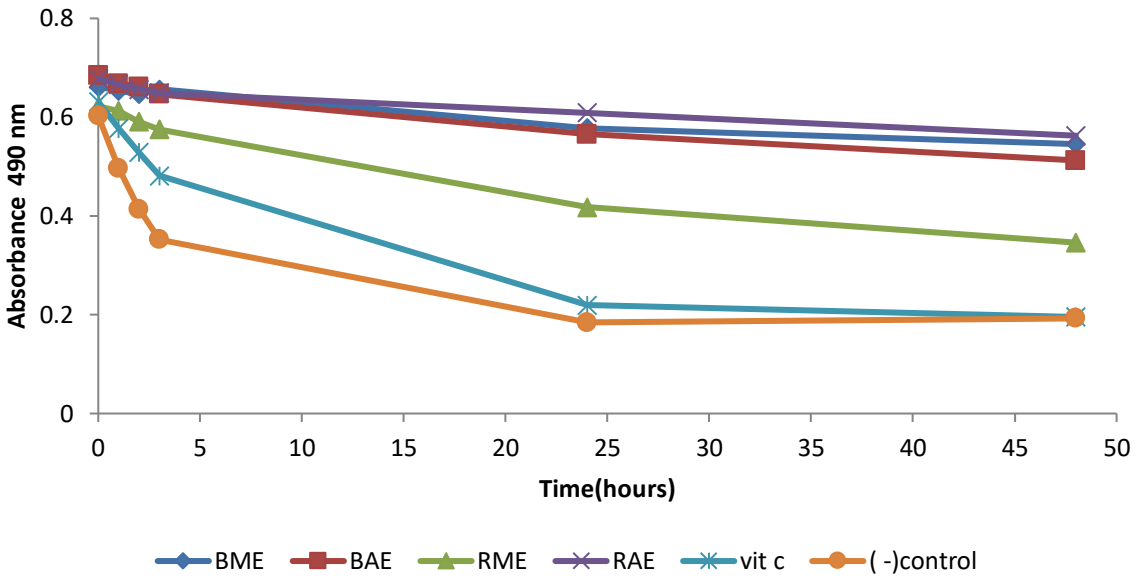

Figure 2: Kinetics of β-carotene bleaching at 490 nm in absence and presence of *Olea europea* extracts. RME: Roots methanolic extract, RAE: Roots aqueous extract, BME: Bark methanolic extract, BAE: Bark aqueous extract. Vit C: Vitamine C. (Each value is the average of three tests).

Diouf et al. [27] explained the behavior of BCB compared with other antioxidant assays; the extraction will solubilize several other classes of molecules in addition to phenolics, like sugars and mineral constituents, which might contain transition rate of generation of free radicals from fatty acids or hydroperoxides. Another explanation about the difference in the data obtained from BCB compared with the other antioxidant assays might be the 'polar paradox, lipophilic antioxidants show greater activity in emulsions, and hydrophilic antioxidants are more efficient than lipophilic antioxidants [28]. This phenomenon explains the very low activity exerted by vitamin C in BCB (0.67±4.81%).

Antihemolytic Activity

Erythrocyte membrane is mostly susceptible to free radicals attack due to its high content of polyunsaturated fatty acids, as well as molecular oxygen transport by haemoglobin [29]. Lipid peroxidation in human erythrocyte membrane mediated by hydrogen peroxide, induces membrane damage and subsequent hemolysis measurable at 540 nm. [30].

This cellular system could have been very useful for the study of the protective effect of the studied extracts against hydrogenperoxide-induced oxidative hemolysis. However, this test could not be performed with the extracts (BAE, RAE and BME). It has been found that these extracts themselves cause significant hemolysis of erythrocytes before exposure to radical attack.

The hemolytic effect of extracts is probably attributed to the presence of saponosids, these results are compatible with the work of Mebirouk-boudechiche and his team [31] who measured primary and secondary compounds in leaves of nine forage arborists, including *Olea europeae*. The results show that olive leaves have hemolytic activity (16.5%) with a saponin content of 19.45 g diosgenin equivalent/kg DM).

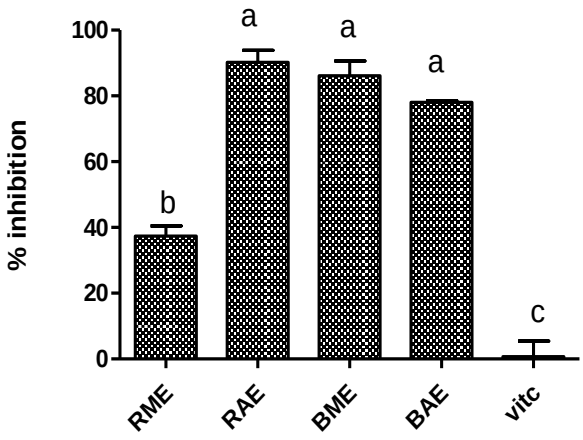

Figure 3: Antioxidant activities of (2 mg/mL) of *Olea europea* extracts using β-carotene /linoleic acid bleaching assay after 48 hours.
RME: Roots methanolic extract, RAE: Roots aqueous extract, BME: Bark methanolic extract, BAE: Bark aqueous extract, Vit C: vitamin C. (Values are the average of three measurements ± SD), bars with different letters indicate activities significantly different ($p \leq 0.01$).

The only extract tested in oxidative hemolysis induced by hydrogen peroxide is the methanol extract of the roots RME. Absorbance and percent inhibition of hemolysis are presented in Fig. 4 and Fig. 5.

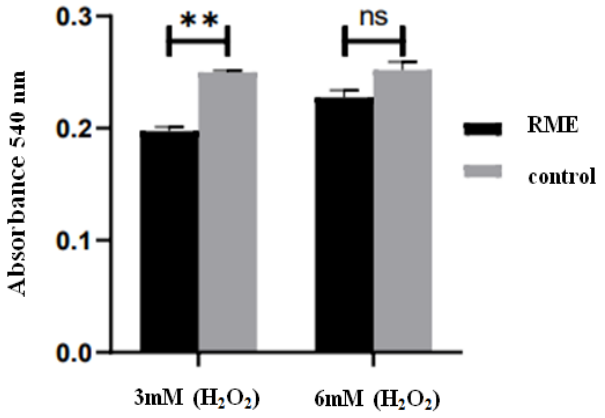

Figure 4: Absorbance of hemoglobinin the presence and absence of RME
Control (CTL): erythrocyte suspension with H_2O_2, without extracts. RME: erythrocyte suspension with H_2O_2 and root methanolic extract. (Values are the average of three measurements ± SD), Significance vs. control: p<0. 01 (**), not significant (ns).

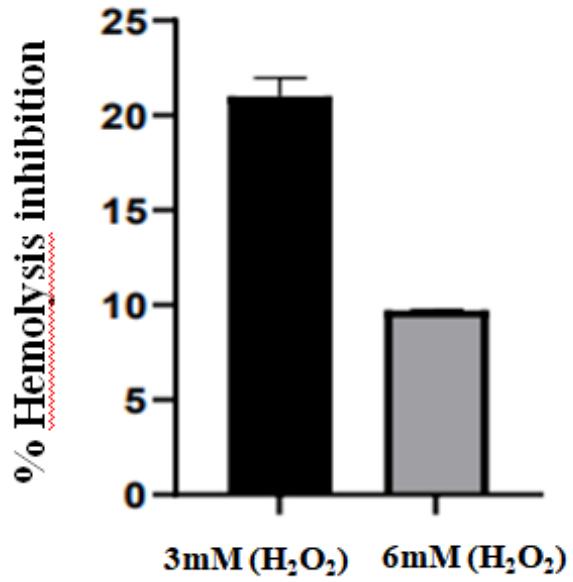

Figure 5: Hemolysis inhibition by RME in presence of hydrogen peroxide

The obtained results show that the RME exerts a significant inhibition (P<0.01) of oxidative hemolysis (21%) induced by 3mM of H_2O_2. However, this inhibition is not significant for 6mMof H_2O_2 (9.72%). These findings are in concordance with the investigation of Lins et al. [23] who studied the effect of hydro methanolic leaf extract of *Olea europea* on APPH-induced hemolysis, the obtained results showed potent inhibition of peroxyl radical-induced hemolysis in dose dependant manner (EC_{50}=11.5±1.5 mg/mL), reduction of TBARS formation (EC_{50}= 38.0±11.7 mg/mL) and hemoglobin oxidation (EC_{50}=186.3± 29.7 mg/mL) in erythrocytes.

This idea is reinforced by the work of Manna and his coworkers [32], who discovered that the hydroxytyrosol in olive oil reduces the hemolysis of human erythrocytes caused by H_2O_2. The antihemolytic activity of RME is likely attributable to its polyphenol content.

According to Bonarska-kujawa et al. [33], phenolic compounds incorporate into the outer hydrophilic layer and have no effect on the fluidity of the hydrophobic part. Thus, the location of polyphenolic compounds in the hydrophilic part of the membrane seems to constitute a protective shield of the cell against other substances, the reactive forms of oxygen in particular.

CONCLUSION

The findings of this study established the ethnopharmacological basis for the use of *Olea europea* in traditional medicine by demonstrating that the bark and the roots have considerable antioxidant properties and contain high amounts of phenolic compounds.

Additionally, our findings show that this plant may be an excellent source of natural antioxidant agents and a suitable candidate for upcoming biomedical uses to support human health with few adverse effects.

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