

Scientific paper

HPLC-DAD Analysis, Antioxidant and Antibacterial Properties of Fruit Extracts from *Pistacia atlantica* Desf.

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Abstract

Pistacia atlantica is commonly used in traditional medicine to treat various diseases in Algeria. This study was carried out to investigate the antioxidant potential and antibacterial properties of fruit extracts. The results indicated various amounts of polyphenols and flavonoids in different extracts. Quercetin, gallic acid, chlorogenic acid and methyl gallate were the dominant constituents in the ethyl acetate extract (EAE) and crude extract (CrE) quantified by HPLC-DAD. EAE was the most active in scavenging DPPH and hydroxyl (OH[•]) radicals, hydrogen peroxide (H₂O₂), reducing power and total antioxidant capacity. All extracts have the ability to inhibit lipid peroxidation. A broad spectrum of antibacterial effects (10.66 to 29.33 mm) was obtained. In addition, the time-kill assay and the MBC/MIC ratio indicated that all extracts were bactericidal against most of the test bacteria and their combination with antibiotics showed remarkable synergistic effect. The findings of this study suggest that medicinal plant is a potential source of natural antioxidant and antibacterial compounds, which could be used where these kinds of activities are warranted.

Keywords: *Pistacia atlantica*; HPLC-DAD; Antioxidant; Antibacterial activity.

1. Introduction

Oxidative stress is a common factor in the genesis of several human diseases such as cancer, Alzheimer's, inflammation, arthritis, diabetes, atherosclerosis and Parkinson's disease.¹ This becomes a major area of research, motivating the scientists to look for more therapeutic plants which provide health benefits for human health. In recent decades, there is more interest in natural antioxidants, which mainly have phenolic structure known for their potential to trap free radicals produced as a result of diverse degradations and disease processes.² These phytochemicals have been recognized as safer with fewer side effects than synthetic oxidants, thus reducing the risk of chronic diseases.³ Furthermore, bacterial infections represent a major public health, that impact millions of individuals every year. Antibiotics are commonly employed to treat microbial infections; however, the high significant genetic variation of harmful pathogens, en-

able them to acquire resistance to these drugs.⁴ This led to a decline in the effectiveness of current antibacterial medications, rendering them less useful or entirely powerless. The increase in multidrug-resistant (MDR) bacteria highlights the necessity of exploring plant-derived products for finding new treatment alternatives and innovative antimicrobial medications. Phenolic compounds are well-known to effectively reduce the damage caused by free radicals and fight off parasites and pathogenic bacteria.⁵

Pistacia atlantica Desf. (Atlas pistachio) commonly known as "Betoum" in Arabic, is one of the plants traditionally used in the treatment of human ailments since ancient times. Atlas pistachio is a tree belonging to the *Anacardiaceae* family, a rare endemic and threatened with extinction species found in the semi-aride, aride and even saharan regions in Algeria.⁶ This tree of 3–5 meters produces small unisexual flowers and spherical red drupe fruits with 5 to 6 mm in size. Different parts of this plant

(fruits and leaves) are widely used in traditional medicine to treat respiratory and digestive systems, throat infections, heal wounds, heart, kidneys, dyspepsia and peptic ulcer.⁷ Scientific research has also revealed many pharmacological activities such as antioxidant, antimicrobial, antihyperglycemic, anti-inflammatory, analgesic, anti-carcinogenic and anti-cholinesterase.⁸ Most of these activities are linked to the content of the secondary metabolites of this plant.⁹ The main objectives of this study were to estimate the phenolic and flavonoid content, and to evaluate the antioxidant potential as well the antibacterial effect of the hydro-methanolic extract and its fractions from *P. atlantica*.

2. Materials and Methods

2.1. Plant Material

Fresh fruits of atlas pistachio (*Pistacia atlantica* Desf. 1977) were collected in September 2023, from Tamanrasset situated in the far southern Algerian Sahara. The plant was identified by Professor Chermat Sabah, Department of Pharmacy, Faculty of Medicine, University Setif1Ferhat Abbas, Algeria. The Fruits were cleaned and allowed to dry at room temperature in a shaded area. Then, dry seeds were ground into a powder using electric mill.

2.2. Extraction Procedure

The hydro-methanolic extract of *P. atlantica* was prepared according to Saffidine *et al.*¹⁰, with slight modification. 500 g of fine powder were macerated with 5000 mL in water-methanol (1: 9) under magnetic agitation for 15 min at 80 °C. The mixture was left to macerate for 7 days. It was then filtered on cotton and filter paper Whatman n° 3. This process was repeated twice again. The filtrates were combined and evaporated at 40 °C in a rotary evaporator under decreased pressure. The residual extract was dried in an oven and stored in refrigerator until use. The percentage yield of this dried crude hydro methanolic extract (CrE) was obtained using the following equation:

$$\text{Yield of extract (\%)} = (w1/w2) \times 100$$

where **w1** is the weight of the extract residue after solvent removal and **w2** is the weight of dried plant powder.

The crude extract (CrE) of *P. atlantica* fruits was fractionated using solvents with increasing polarity, including petroleum ether, ethyl acetate. 25 g of dry extract was suspended in 200 mL of boiling distilled water and let to decant in the refrigerator for 30 min, then filtered through filter paper. First, the aqueous solution was fractionated with petroleum ether to remove lipids, then with ethyl acetate. Three fractions were obtained after this process: petroleum ether (PEE) and ethyl acetate (EAE) and residual aqueous fraction (AqE). These fractions were al-

lowed to dry at 37 °C in an oven after being evaporated at 40 °C under reduce pressure using a rotary evaporator. After that, the dried fractions were stored in a refrigerator until further tests.

2.3. Determination of Total Phenolic Content (TPC)

P. atlantica extracts were subjected to Folin-Ciocalteu (FC) method to measure the total phenolic content (TPC).¹¹ This process is based on the reaction of the phenol compounds with the FC reagent (complex of phosphomolybdic and phosphotungstic acids) and the blue color that results is correlated with amount of polyphenols. 200 µL of extracts or Gallic acid as standard (0–200 µg/mL) was mixed with 1 mL of FC reagent (1/10). After 4 min, 800 µL of sodium carbonate (7.5%) was added and the mixture was incubated for 2 hours at room temperature. Then, the absorbance was measured at 765 nm. TPC was expressed in milligrams of Gallic Acid Equivalent per gram of dry extract (mg GAE/g DE).

2.4. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) of different extracts was determined using the aluminum chloride (AlCl₃) assay.¹² In the presence of aluminum chloride, free hydroxyl groups of flavonoids gave a yellowish complex which is proportional to the quantity of flavonoids presents in the extract. 1 mL of samples or quercetin (0–40 µg/mL) as standard was mixed with 1 mL of AlCl₃ solution (2%). The mixture was incubated at room temperature for 10 min and the absorbance was measured at 430 nm. The result was expressed as micrograms of quercetin equivalent per milligrams of dry extract (µg EQ/mg of extract).

2.5. HPLC-DAD Analysis

Characterization and quantification of phenolic compounds in CrE and EAE extracts of *P. atlantica* was carried out by High performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) method used in this study and was adapted from the protocol established by Gheraibia *et al.* (2020),¹³ using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of solvent A (water) and solvent B (0.05% trifluoroacetic acid in acetonitrile) at a flow rate 0.9 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1–11 min (75% A); 11–18 min (60% A); 18–22 min (82% A); 22–24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µL for samples and standards solutions and the column temperature was maintained at 40 °C.

2. 6. Antioxidant Activity

2. 6. 1. DPPH Scavenging Assay

DPPH is a stable free radical commonly used to assess the radical scavenging activity of plant extract due to its high sensitivity. This assay is based on the measurement of the capacity of the extracts to scavenge this free radical. In the presence of antioxidant, the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) loses its characteristic dark purple color and is reduced to yellow diphenylpicryl hydrazine. The method consisted in mixing 50 μ L of different concentrations of extracts or butyl hydroxytoluene (BHT) as standard with 1250 μ L of DPPH solution (0.004% in methanol).¹⁴ After incubation for 30 min in the dark at room temperature, absorbance was measured at 517 nm. The synthetic antioxidant butyl hydroxytoluene (BHT) was used as positive control. Antiradical activity (AR) was calculated using the following equation:

$$AR (\%) = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the solution containing only DPPH radical solution. A_1 is the absorbance of the DPPH solution in the presence of the sample. The IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radical.

2. 6. 2. Hydroxyl Radical (HO $\cdot$) Scavenging Assay

This assay was performed based on the method described by Mayouf¹⁵, with few modifications. The reaction mixture consists of 150 μ L of extracts or standard (vitamin C) at different concentrations, mixed with 300 μ L of a stock solution containing ferric sulfate ($FeSO_4$ 9 mM) and hydrogen peroxide (0.3%). The reaction between $FeSO_4$ and H_2O_2 lead to the generation of hydroxyl radicals according to the Fenton reaction. The mixture was incubated for 15 min at 32 $^{\circ}C$. Then 75 μ L of salicylic acid (20 mM) was added to the reaction mixture, which was incubated again for 15 min at 32 $^{\circ}C$. The absorbance was measured at 562 nm.

2. 6. 3. Hydrogen Peroxide (H_2O_2) Scavenging Assay

The capacity of the extracts to scavenge H_2O_2 is based on the reaction of ferrous ion (Fe^{+2}) with 1,10-phenanthroline, which forms red-orange tri-phenanthroline complex, indicating a potent radical scavenging of H_2O_2 in the media. This assay was performed by mixing 63 μ L of ferrous ammonium sulfate (1 mM) with 375 μ L of different concentrations of extracts or standard.¹⁶ Then, 16 μ L of hydrogen peroxide (5 mM) was added to the mixture and incubated at room temperature in the dark for 5 min. After that, 375 μ L of 1,10-phenanthroline (1 mM) was added and incubated again for 10 min. The absorbance of the

solution was read at 510 nm. Hydrogen peroxide scavenging activity (HPSA) was calculated according to the following equation:

$$HPSA (\%) = (A_{\text{sample}} / A_{\text{control}}) \times 100$$

A sample: absorbance of the sample.

A control: absorbance of the control.

2. 6. 4. Reducing Power Assay

This assay is based on the capacity of the extracts to reduce the ferric iron of the ferricyanide complex- Fe^{3+} to ferrous iron- Fe^{2+} of blue color. 100 μ L of different dilutions of extracts or standard were added to 100 μ L phosphate buffer (pH 6.6) and 100 μ L potassium ferricyanide (1%). The mixture was incubated at 50 $^{\circ}C$ for 20 min, followed by addition of 250 μ L of trichloroacetic acid TCA (10%) and centrifuged for 10 min at 3000 rpm. Then, 250 μ L of the upper layer solution was mixed with 250 μ L of distilled water and 500 μ L of 0.1% ferric chloride solution ($FeCl_3$). The absorbance was determined at 700 nm against a blank. Ascorbic acid (vitamin C) was used as the standard. The effective concentration (EC_{50}) at which the absorbance reaches 0.5, was used as reducing capacity potential of the extract.¹⁰

2. 6. 5. Total Antioxidant Capacity (TAC)

Total antioxidant activity of plant extracts was estimated by phosphomolybdenum assay.¹⁷ When antioxidants in the sample reduce the molybdenum complex, a green-colored complex is produced. 100 μ L of plant extract or Trolox as standard in different concentrations were added to 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was incubated for 90 min at 95 $^{\circ}C$. After the solutions had cooled to room temperature, the absorbance was then measured at 695 nm.

2. 6. 6. Lipid Peroxidation Inhibition

The ferric thiocyanate (FTC) method was used to determine the antioxidant property of extracts in lipid like-system.¹⁸ The ferrous iron (Fe^{2+}) is converted to ferric iron (Fe^{3+}) by a hydroperoxides (LOOH) produced when linoleic acid is oxidized. The resultant ions (Fe^{3+}) then combine with thiocyanate to form a red complex that has a maximum absorbance at 500 nm. The emulsion was prepared by mixing 155 μ L of linoleic acid and 0.2804 g of Tween 20 in 50 mL of phosphate buffer (0.02 M at pH 7.0). After that, 0.5 mL of extract solution or standard (BHT) was mixed with 2.5 mL of linoleic acid emulsion and incubated for 5 days at 37 $^{\circ}C$. A volume (0.1 mL) of the reaction mixture was mixed with 4.7 mL of ethanol (75%) and 0.1 mL of ammonium thiocyanate (30%). Then, 0.1 mL

FeCl₃ (0.02 M in 3.5% HCl) was added. The absorbance at 500 nm was measured 3 min later after adding ferrous chloride to the reaction mixture. This procedure was repeated each day. The inhibition of lipid peroxidation was determined for each day using the following formula:

$$\% \text{ inhibition} = (\text{Ac} - \text{As} / \text{Ac}) \times 100$$

where **Ac** is the absorbance of the control reaction and **As** is the absorbance of the sample.

2. 6. 7. Thiobarbituric Acid (TBA) Method

This assay was conducted at the last day of the FTC method to detect the formation of the malondialdehyde (MDA), the final product formed during the oxidation of linoleic acid. MDA forms a pink complex with TBA.¹⁹ A volume of 1 mL from the incubated combination of linoleic acid emulsion and extract was added to 2 mL of a 20% solution of trichloroacetic acid (TCA) and 2 mL thiobarbituric acid solution TBA (0.67%). This resulting mixture was subjected to a boiling water bath for 10 min, and once it had cooled down, it was centrifuged at 3000 rpm for 20 min. The supernatant's absorbance was measured at 532 nm.

2. 7. Antibacterial Activity

2. 7. 1. Bacterial Strains

The antibacterial activity was evaluated using referenced strains ATCC (American Type Culture Collection): *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and clinical strains: *Escherichia coli* 982, *Pseudomonas aeruginosa* 982, *Klebsiella pneumoniae* 982, *Staphylococcus aureus* 995, *Streptococcus pneumoniae* 990 acquired from Laboratory of Bacteriology at Setif hospital. Other referenced strains: *Bacillus cereus* (ATCC 10876), *Salmonella typhimurium* (ATCC 13311), *Enterococcus faecalis* (ATCC 49452), *Citrobacter freundii* (ATCC 8090), *Klebsiella pneumoniae* (ATCC 700603), *Listeria monocytogenes* (ATCC 15313) and *Proteus mirabilis* (ATCC 35659) obtained from Laboratory of Natural Substances at the University of Tlemcen, Algeria.

2. 7. 2. Agar Well Diffusion Assay

The antibacterial susceptibility of the tested bacteria to different extracts of *P. atlantica* was evaluated using the agar well diffusion method.²⁰ Mueller-Hinton agar medium was autoclaved and poured into the Petri dishes. The suspension of each strain from young colonies of 18 to 24 hours was made in nutrient broth and its concentration was adjusted to 0.5 McFarland (10⁸ CFU/mL). After that, the inoculums of various bacteria were spread over the surface of Mueller Hinton agar, and four wells of 6 mm in

diameter were punched off into the agar medium with sterile cork borer. Three of these wells were filled with 50 µL of extract and the fourth well was poured with 50 µL of sterile distilled water as negative control. The plates were kept for 1 hour at room temperature to allow the diffusion of extracts, and then incubated at 37 °C for 24 h. The results were obtained by measuring the diameter of inhibition zones around the wells. Standard antibiotic discs Cefotaxime (CTX) was used as positive control against *E. coli*, *K. pneumoniae*, *L. monocytogenes*, *C. freundii*. Imipenem (IPM) was used for *B. cereus*, Amoclan (AMC) for *E. faecalis* and *P. mirabilis*. Bactrim (SXT), Oxacillin (OXA), Ceftazidime (CAZ) were tested respectively on *S. typhimurium*, *S. aureus* and *P. aeruginosa*.

2. 7. 3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The minimum inhibitory concentration (MIC) was assessed for plant extracts exhibiting antibacterial activity, by broth microdilution method using 96 wells microplates.²¹ A two-fold serial dilution of the extract was prepared in the 10 wells of sterile microplate containing 25 µL Mueller Hinton nutrient broth. The inoculums of the bacterial strains were prepared from fresh cultures of 18 to 24 hours. The turbidity was adjusted to 0.5 McFarland (10⁸ CFU/mL) and then these suspensions were diluted (1/10). 5 µL of it were inoculated into all wells except the 11th well which is considered as negative control and the 12th as growth positive control. Subsequently, 70 µL of Mueller Hinton medium was added to 96 wells. The microplates were incubated at 37 °C for 24 hours and MIC was defined as the lowest concentration of the extract displaying no visible growth of the tested microorganism. To determine MBC, 2 µL from each well that did not exhibit any growth was inoculated in parallel streaks of 3 cm on Mueller-Hinton agar plates, and then we incubated at 37 °C for 24 hours. The number of bacterial colonies on the streaks was compared with that on the control plate, which contained the streaks of the diluted inoculums (10⁻¹, 10⁻², 10⁻³ and 10⁻⁴) of the bacteria. MBC was defined as the lowest concentration of the extract at which the bacterial growth was totally inhibited. The ratio between MBC and MIC was calculated. When this MBC/MIC is less than 4, the extract is considered as bactericidal against the tested strains; while a ratio above 4 means that the extract is bacteriostatic.

2. 7. 4. Time-dependent Antibacterial Activity

The impact of contact time on the antibacterial activity of *P. atlantica* extracts was determined using the spread plate method.²² The bacterial strain suspensions were prepared from a fresh culture incubated for 18 to 24 hours, with a concentration of 10⁶–10⁷ CFU/mL. 100 µL of each

bacterial suspension were incubated with 10 mL of the extract dissolved in nutrient broth at 200 rpm in a shaker incubator for 2 and 4 hours. After the period incubation, series of 10-fold dilutions were carried out on the combination of the suspension and extract. Then, 100 µL of each dilution was spread onto agar plates, which were incubated for 24 hours at 37 °C. The results were expressed by counting the colonies and comparing them with the control plate to estimate the inhibition activity. The following formula was used to calculate the percentage loss of bacterial viability (LV):

$$LV (\%) = (Nc - Nt) / Nc * 100$$

Nc: the number of colonies in the control plates.

Nt: the number of colonies in the treated plates.

2. 7. 5. Synergistic Antibacterial Assay

To assess the synergistic antibacterial activity, *P. atlantica* extracts were combined with standard antibiotics using disc diffusion method. 20 µL of each extract was dispensed on the antibiotic disk, and then placed on the surface of Mueller-Hinton agar inoculated with the tested bacteria. The plates were incubated at 37 °C for 24 hours and the zones of inhibition produced by the single standard or by the plant extract in combination with standard antibiotics were measured.²¹ Synergism is interpreted as when zone of combination > zone of standard antibiotic; if zone of combination = zone of standard antibiotic, it means indifference and if zone of combination < zone of standard antibiotic, it is interpreted as antagonism.

2. 8. Statistical Analysis

All the tests were performed in triplicates. Results are expressed as mean ± standard deviation. Statistical evaluation was conducted with Graph Pad 8. ANOVA one way was employed to establish basic comparison. The differences were statistically considered significant at $P < 0.05$

3. Results and Discussions

3. 1. Yield, Total Phenols and Flavonoids Content of *P. atlantica* Extracts

In the present study, the yield percentage of the different extracts, total phenolic (TPC) and flavonoids (TFC) content are presented in table 1. The result of extraction yield of *P. atlantica* fruits showed that the maximum percent of 67.32 and 22.04% was obtained by aqueous (AqE) and petroleum ether (PEE) extracts, followed by crude (CrE) and ethyl acetate (EAE) extracts.

Table 1. Yield (%), total phenolic and flavonoids content (TPC and TFC) of fruit extracts from *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate (EAE) and aqueous (AqE) fractions. Values are mean ± SD (n = 3). Different superscript letters indicate significant difference within column ($P < 0.05$).

Extract	Yield %	TPC (mg GAE/g)	TFC (µg QE/mg)
CrE	11.58 ^c	145.45±3.49 ^b	35.05±0.04 ^b
PEE	22.04 ^b	45.60±3.404 ^c	11.62±0.59 ^d
EAE	4.08 ^d	266.34±15.50 ^a	62.7±0.42 ^a
AqE	67.32 ^a	115.64±3.244 ^d	12.77±0.07 ^c

Total phenolic (TPC) and flavonoid (TFC) contents showed significant differences in plant fruit extracts. Thus, the highest amount of TPC and TFC was recorded with EAE (266.34 ± 15.50 mg GAE/g and 62.7 ± 0.42 µg QE/mg), followed by CrE, AqE and PEE.

The differences in the yield of various extracts may be due to the characteristics of the solvent or the nature of phenolic compounds that have being extracted.²³ Moreover, the synthesis of these compounds is influenced by several factors including light, temperature, humidity and nutrient availability.²⁴ Our results are in agreement with several other studies that demonstrate that *P. atlantica* fruit extracts contain a significant amount of these phenolic compounds.^{25,26}

3. 2. HPLC Analysis

The identification and quantification of *P. atlantica* phenolic compounds were performed using HPLC-DAD analysis. The amount and retention time of the identified compounds are presented in table 2 and supplementary information.

15 compounds were identified in each extract with various concentrations. Quercetin (19818.42 µg/g), gallic acid (12871.49 µg/g), chlorogenic acid (3960.28 µg/g) and methyl gallate (3619.76 µg/g) were the dominant constituents in CrE. However, other compounds including rutin, ellagic acid, coumaric acid, syringic acid, caffeic acid and kaempferol were present in moderate concentration, while lowest concentration was showed with vanillin, naringenin, rosmarinic acid, daidzein and ferulic acid. EAE was characterized by high amount of gallic acid (47539.53 µg/g), methyl gallate (31376.82 µg/g), quercetin (28824.60 µg/g) and chlorogenic acid (12184.13 µg/g); followed by ellagic acid, coumaric acid, syringic acid, naringenin, vanillin and daidzein with moderate concentration. Many studies have shown that these phenolic compounds have beneficial effects on human health, owing to their biological activities including antioxidant, anti-inflammatory and antibacterial activities.²⁷

Table 2. HPLC analysis of phytoconstituents in crude (CrE) and ethyl acetate (EAE) extracts of *P. atlantica*. RT: retention time, C: concentration.

Peak	Compounds	CrE		E-AE	
		RT (mn)	C (µg/g)	RT (mn)	C (µg/g)
1	Gallic acid	3.535	12871.49	3.531	47539.53
2	Chlorogenic acid	4.267	3960.28	4.245	12184.13
3	Catechin	–	0.00	–	0.00
4	Methyl gallate	5.386	3619.76	5.382	31376.82
5	Coffeic acid	5.715	314.12	–	0.00
6	Syringic acid	6.151	354.21	6.151	2858.16
7	Rutin	6.841	752.98	–	0.00
8	Ellagic acid	7.194	630.08	7.174	6875.11
9	Coumaric acid	8.314	457.08	8.309	3677.68
10	Vanillin	9.124	163.18	9.083	479.28
11	Ferulic acid	9.440	35.28	9.436	156.42
12	Naringenin	10.122	156.56	10.124	1549.28
13	Rosmarinic acid	10.035	62.71	11.699	80.19
14	Daidzein	15.659	42.51	16.176	225.04
15	Quercetin	17.437	19818.42	17.427	28824.60
16	Cinnamic acid	–	0.00	19.041	6.32
17	Kaempferol	20.307	256.78	20.295	23.83
18	Hesperetin	–	0.00	21.088	58.53

3. 3. Antioxidant Activities

3. 3. 1. DPPH Scavenging Assay

The results of DPPH radical scavenging activity are illustrated in table 3. Among the tested extracts, EAE exhibited the strongest DPPH radical scavenging activity with very low IC₅₀ (5.59 ± 0.27 µg/ml) more effective than BHT as standard. CrE and AqE also showed a considerable activity. In contrast, PEE displayed the lowest capacity.

Table 3. Radical scavenging activities of different extracts from *P. atlantica* fruits: crude extract (CrE), petroleum ether (PEE), ethyl acetate (EAE) and aqueous (AqE) fractions; and standards (BHT and Vitamin C). Results were expressed as the mean ± SD (n = 3). ns: no significant difference, ***: P < 0.001, ****: P < 0.0001.

Extracts	DPPH IC ₅₀ µg/ml	OH· IC ₅₀ mg/ml	H ₂ O ₂ IC ₅₀ µg/ml
CrE	22.35±0.12 ^{ns}	0.46 ± 0.02 ^{***}	158.74±1.92 ^{****}
PEE	439.04±5.79 ^{****}	3.91 ± 0.04 ^{****}	745.7±17.88 ^{****}
EAE	5.59±0.27 ^{***}	0.50 ± 0.02 ^{****}	20.56±5.84 ^{ns}
AqE	48.48±1.13 ^{****}	1.55 ± 0.04 ^{****}	121.14 ± 6.08 ^{****}
BHT	20.00± 0.11	–	–
Vitamin C	–	0.29 ± 0.01	36.17±5.49

Our results showed that *P. atlantica* from Algeria is more active in scavenging action than the same plant from different area in the world,^{26,28,29} and other species including *P. vera*,³⁰ *P. lentiscus*,³¹ and *P. khinjuk*.³²

3. 3. 2. Hydroxyl Radical (HO·) Scavenging Assay

The Hydroxyl radicals (OH·) are formed during the metabolic process in cells, known for their extreme reac-

tivity and capability to interact various biomolecules such as lipids, proteins and DNA, causing harm to the organisms. Hence, human health relies on the removal of these free radicals.³³ According to the table 3, CrE and EAE showed the highest scavenging of hydroxyl radical (OH·) with IC₅₀ of 0.46 ± 0.02 and 0.50 ± 0.02 mg/mL, respectively. However, AqE and PEE exhibited the lowest activities. These results are in accordance with the findings obtained for the different extract from the leaves of *P. atlantica*,³⁴ and are more notable than those found in other species such as *P. vera*,³¹ and *P. lentiscus*,³² and *P. khinjuk*.³⁵

3. 3. 3. Peroxide (H₂O₂) Scavenging Assay

Hydrogen peroxide itself exhibit a low reactivity, due to its capacity to penetrate cell membrane, it can oxidize various intracellular molecules. Furthermore, its reaction with transition metals like iron produces more reactive species such as hydroxyl radicals.³⁶ This indicates the importance of removing hydrogen peroxide by the antioxidant defense systems. In hydrogen peroxide scavenging assay, EAE showed a potent scavenging capacity with an IC₅₀ = 20.56 ± 5.84 µg/mL, exceeding both the standard and the other extracts (table 3). These results represent the first report on hydrogen peroxide scavenging activity of *P. atlantica* fruits extracts, based on our knowledge. Our findings showed that *P. atlantica* extracts possessed a significant antioxidant potential, which may be explained by the presence of phyto-constituents, especially phenolic acids and flavonoids. These phenolic compounds are well-known for their biological properties,^{37,38} acting as radical quenchers by donating a hydrogen atom or through electron transfer.³⁹

3. 2. 4. Reducing Power Assay

Reducing power assay results are illustrated in figure 1 and indicated that all the extracts possessed the ability to reduce ferric iron. EAE had the strongest reducing activity ($EC_{50} = 7.11 \pm 0.10 \mu\text{g/ml}$), which was similar to that of the standard vitamin C ($5.87 \pm 0.06 \mu\text{g/ml}$), followed by CrE and AqE. The reducing power of these extracts could be related to the presence of the phenolic compounds which act as electron donor in the reduction process.^{40,41}

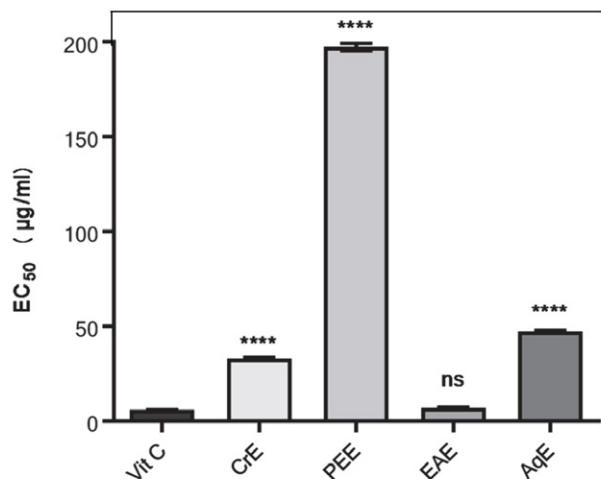


Figure 1. EC_{50} in reducing power of *P. atlantica* extracts: crude extract (CrE), petroleum ether (PEE), ethyl acetate (EAE) and aqueous (AqE) fractions; and the standard (vitamin C). values are expressed as mean $\pm$ SD of triplicate. ns: No significant difference, ****: $P < 0.0001$.

Our results are in agreement with those obtained by Belyagoubi *et al.*⁴⁰ and Benmohamed *et al.*²⁶, but were more effective than those of the study conducted on leaves by Zerkani *et al.*²⁵

3. 2. 5. Total Antioxidant Capacity (TAC)

This assay is a quantitative method used to evaluate the sample's ability to reduced phosphate- Mo (VI) to phosphate-Mo (V) through electron or proton donation, during a prolonged incubation at higher temperatures and under acidic conditions.^{42,43} According to the results presented in figure 2, EAE showed the strongest antioxidant capacity ($EC_{50} = 18.23 \pm 0.23 \mu\text{g/mL}$) than the other fractions and the standard Trolox. This can be related to the high flavonoids content in this extract. A moderate TAC was exhibited by CrE and AqE.

These results are comparable to that of the study conducted on *P. lentiscus* leaves and seeds.⁴⁴ Previous studies have indicated that this plant contain a considerable amount of kaempferol, myricetin, quercetin and its derivatives,⁴⁵ which exist in our extracts and these compounds are known for their great capacity as antioxidant.

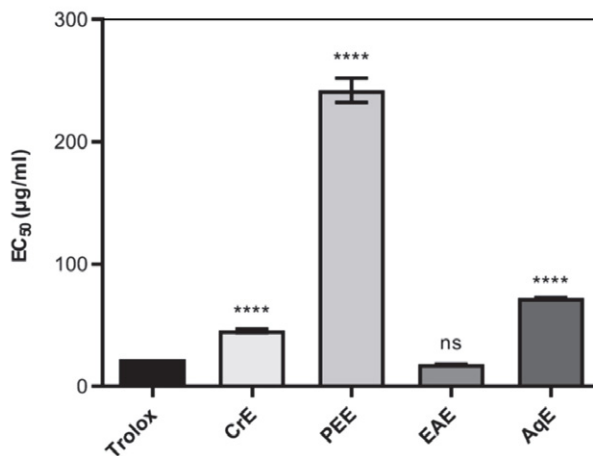


Figure 2. EC_{50} in total antioxidant capacity (TAC) of fruit extracts from *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions; and standard (Trolox). Results are mean of 3 replicates values. ns: No significant difference, ****: $P < 0.0001$.

3. 2. 6. Lipid Peroxidation Inhibition

Lipid peroxidation is a process in which free radicals attack poly-unsaturated lipids in cell membranes, leading to cell damage, which can be inhibited by antioxidants. In this assay, peroxides are formed during the oxidation of linoleic acid and have the ability to oxidize Fe^{2+} to Fe^{3+} . This later forms a red complexe with thiocyanate.⁴⁶ The effect of the *P. atlantica* extracts on lipid peroxidation inhibition obtained after five days is represented in figure 3 and showed that EAE and PEE have the highest capacity to inhibit lipid peroxidation with percentage inhibition of $82.86 \pm 0.31\%$ and $80.11 \pm 2.54\%$, respectively, and was comparable to that of the standard BHT ($81.10 \pm 0.68\%$), followed by CrE ($74.83 \pm 1.5\%$).

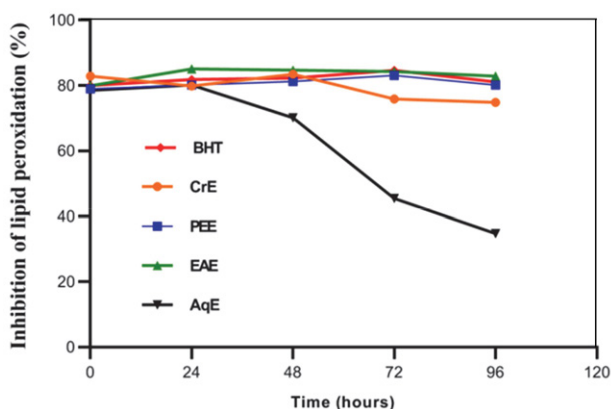


Figure 3. Kinetic of lipid peroxidation inhibition of fruit extracts from *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions; and the standard (BHT), at a concentration of 2 mg/mL.

The potent activities of these extracts may be due to the quantity and the quality of extracts compounds, which neutralize free radicals and convert them into more stable

form. This stops the radical chain reactions,⁴⁷ in case of PEE, its antioxidant capacity may be linked to the presence of lipophilic antioxidants such as fatty acid and terpenoids.⁴⁸ These compounds are known for their ability to scavenge free radicals, which can provide protection against lipid peroxidation.⁴⁹

3. 2. 7. Thiobarbituric Acid (TBA) Method

The end product of lipid peroxydation, MDA serves as a biomarker in this experiment and its concentration directly reflects the extent of lipide damage triggered by oxidative stress. The data of this assay are shown in figure 4. EAE was the most effective extract in inhibiting MDA formation ($93 \pm 1.14\%$) than BHT ($89.37 \pm 0.19\%$), followed by PEE ($88.29 \pm 0.32\%$), and CrE ($87.59 \pm 0.25\%$).

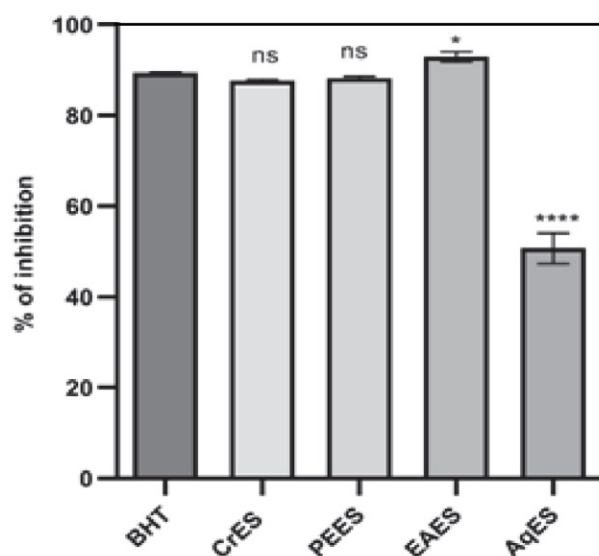


Figure 4. The percent inhibition of MDA formation by *P. atlantica* extracts: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions; the standard (BHT). Values are % means $\pm$ SD ($n = 3$). ns: no significant difference. ****: $P < 0.0001$.

These obtained activities for *P. atlantica* fruits, were more significant than those reported for leaves extracts of the same plant,³⁴ and *P. khinjuk* extracts.³⁵

3. 3. Antibacterial Activity

3. 3. 1. Well Diffusion Method

The results of the antibacterial activity of *P. atlantica* extracts screened on 15 different bacterial strains using the well diffusion method are indicated in the table 4. All extracts exhibited a broad spectrum of antibacterial activity against both gram-positive and gram-negative pathogenic bacteria with inhibition zones ranging from 10.66 to 29.33 mm. EAE demonstrated the strongest activity against *S.*

aureus, *P. aeruginosa* 982, *S. typhimurium*, *S. aureus* 995, *B. cereus*, *E. faecalis*, *C. freundii* and *P. mirabilis*. These antibacterial activities were comparable to that of the standard antibiotics. Moreover, clinical stains: *S. pneumoniae* 990, *K. pneumoniae* 982, and *E. coli* 982 were only sensitive to EAE and their inhibition zones varied from 11 to 14 mm. On the other hand, CrE and PEE showed the highest inhibition zones against *K. pneumoniae* and *S. aureus* 995, respectively; and moderate inhibition zones on *S. aureus*, respectively. However, AqE exhibited the lowest activity.

Our results showed strongest activities against several bacteria species than that of obtained by Benmohamed *et al.*²⁶, and Benhamou *et al.*⁴⁹, and were comparable to those reported for *P. chinensis*,⁵⁰ and *P. khinjuk*.⁵²

Chemical investigations of various *Pistacia* species have revealed their richness in phenolic compounds,^{28,53} which are active against a wide range of microorganisms.^{54,55} This may be due to their interaction with bacteria cell membrane^{56,57} or the inhibition of enzymes essential for various metabolic processes.⁵⁸

3. 3. 2. Time-dependent Antibacterial Activity

The results of the effect of contact time on the bacterial viability are illustrated by table 5 and showed potent and various antibacterial activities against different bacterial strains. The rates of growth inhibition were expressed in percentage with values ranging from 79.39 to 99.98% and from 22.58 to 100% after the 2 and the 4 hours of incubation period, respectively. Thus, the most active extract was EAE against 11 bacterial strains and the mortality rates varied from 87.41 to 99.86% after 2 hours of contact and remained stable against most of the strains, ranging from 88.48 to 99.98% after 4 hours at a concentration of 10 mg/mL. This extract was bactericidal on almost species.

Significant and similar rates were also recorded for CrE and PEE at a concentration of 20 mg/mL, after 2 and 4 hours of contact time. These extracts were bactericidal against four bacterial strains. Conversely, AqE showed a high mortality rate after 2 hours against *S. typhimurium* and *K. pneumoniae*, which significantly decreased after 4 hours. The strong antibacterial activity of EAE is due to its richness in bioactive compounds such as phenolic acids and flavonoids whose potential antibacterial effect was demonstrated.

3. 3. 4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

After screening antibacterial activity using the agar-well diffusion assay, the most active extracts were further tested to evaluate their MIC and MBC. As shown in table 6, the MICs varied from 0.02 to 6.25 mg/mL and MBCs ranged from 0.19 to 50 mg/mL. The EAE was the most active with low MIC values on *S. pneumoniae* 990, *B. cereus*,

Table 4. Antibacterial activity of fruit extracts from *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions; and the standard antibiotics. The results were expressed as mean $\pm$ SD of triplicate determinations.

Bacterial strains	Inhibition zone (mm)				
	CrE	PEE	EAE	AqE	antibiotics
Ec	–	–	12.00 $\pm$ 1.00	–	28.00 (CTX)
Pa	–	–	14.00 $\pm$ 1.41	–	11.00 (CAZ)
Sa	16.00 $\pm$ 1.00	14.5 $\pm$ 0.70	29.33 $\pm$ 1.15	–	27.00 (OXA)
Bc	12.66 $\pm$ 2.08	17.5 $\pm$ 0.70	18.5 $\pm$ 3.53	–	31.50 (IPM)
St	–	–	20.00 $\pm$ 2.82	12.33 $\pm$ 0.57	25.00 (SXT)
Ef	–	–	17.66 $\pm$ 0.57	–	22.50 (AMC)
Cf	–	–	17.66 $\pm$ 2.08	–	35.00 (CTX)
Kp	23.00 $\pm$ 7.93	22.5 $\pm$ 3.35	16.50 $\pm$ 2.21	12.33 $\pm$ 2.51	22.00 (CTX)
Pm	13.33 $\pm$ 1.52	–	17.00 $\pm$ 1.00	–	29.00 (AMC)
Ec 982	–	–	11.00 $\pm$ 1.41	–	32.00 (CTX)
Pa 982	–	–	21.00 $\pm$ 1.00	–	23.00 (CAZ)
Kp 982	–	–	13.50 $\pm$ 0.70	–	(CTX)
Sa 995	16.33 $\pm$ 1.52	17.16 $\pm$ 2.02	19.33 $\pm$ 1.15	–	22.00 (OXA)
Sp 990	–	–	14.00 $\pm$ 1.41	–	

Ec: *E. coli*, Pa: *P. aeruginosa*, Sa: *S. aureus*, Bc: *B. cereus*, St: *S. typhimurium*, Ef: *E. faecalis*, Cf: *C. freundii*, Kp: *K. pneumoniae*, Pm: *P. mirabilis*, Ec982: *E. coli* 982, Pa 982: *P. aeruginosa* 982, Kp982: *K. pneumoniae* 982, Sa 995: *S. aureus* 995, Sp990: *S. pneumoniae* 990.

Table 5. Loss of bacterial viability (%) with *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions, after 2 and 4 hours of incubation.

Bacterial Strains	CrE		PEE		EAE		AqE	
	2 hours	4 hours	2 hours	4 hours	2 hours	4 hours	2 hours	4 hours
Ec	–	–	–	–	93.76 $\pm$ 1.25	97.12 $\pm$ 0.65	–	–
Sa	99.98 $\pm$ 0.08	99.99 $\pm$ 0	92.65 $\pm$ 0.24	89.82 $\pm$ 1.27	99.65 $\pm$ 0.04	99.88 $\pm$ 0.02	–	–
Bc	–	–	99.88 $\pm$ 0.09	99.87 $\pm$ 0.03	97.18 $\pm$ 0.65	94.69 $\pm$ 0.68	–	–
St	–	–	–	–	93.47 $\pm$ 1.17	93.65 $\pm$ 7.92	89.84 $\pm$ 1.06	22.58 $\pm$ 0.44
Ef	–	–	–	–	95.02 $\pm$ 0.27	94.23 $\pm$ 0.50	–	–
Cf	–	–	–	–	99.58 $\pm$ 0.04	99.67 $\pm$ 0.04	–	–
Kp	99.07 $\pm$ 1.29	99.60 $\pm$ 0.55	90.77 $\pm$ 1.07	95.88 $\pm$ 0.24	96.35 $\pm$ 1.18	95.31 $\pm$ 0.82	79.39 $\pm$ 6.46	28.78 $\pm$ 3.50
Pm	99.11 $\pm$ 0.16	100.00 $\pm$ 0	–	–	94.22 $\pm$ 0.53	99.35 $\pm$ 0.08	–	–
Pa 982	–	–	–	–	95.45 $\pm$ 0.26	94.94 $\pm$ 0.79	–	–
Kp 982	–	–	–	–	87.41 $\pm$ 1.03	88.48 $\pm$ 3.45	–	–
Sa 995	99.69 $\pm$ 0.41	99.98 $\pm$ 0.08	99.84 $\pm$ 0.01	99.48 $\pm$ 0.04	99.86 $\pm$ 0.05	99.98 $\pm$ 0.01	–	–
Sp 990	–	–	–	–	95.53 $\pm$ 1.15	95.60 $\pm$ 1.93	–	–

S. typhimurium, *C. freundii*, and *P. aeruginosa* 982, *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. aureus* 995. Similarly, the CrE displayed a significant MICs for *K. pneumoniae* and *B. cereus*. However, PEE and AqE displayed moderate MIC against the tested bacteria. Our results were better than those reported by Rigane⁵⁸, and Hasheminya.⁵⁹

On the other side, the bacteriostatic and bactericidal nature of *P. atlantica* extracts against the selected microorganisms was appreciated through the MBC/MIC ration. As shown in table 5, EAE was bactericidal against 7 strains: *E. coli*, *S. aureus*, *B. cereus*, *P. mirabilis*, *E. coli* 982, *K. pneumoniae* 982 and *S. aureus* 995. Similarly, the CrE and PEE demonstrated a bactericidal effect against four bacterial strains. However, AqE displayed bactericidal activity only on *K. pneumoniae*.

3. 3. 5. Synergistic Antibacterial Assay

In our study, the plant extracts combined with antibiotic exhibited a significant synergistic ability to inhibit the growth of microorganisms and inhibition zones enhanced between 3 and 13 mm (table 7). EAE showed strong synergistic effect against various bacterial strains, followed by CrE and PEE. However, AqE, has a significant synergistic only on *K. pneumoniae*.

The findings revealed a potent synergy of the plant extracts with standard antibiotics, which could provide new possibilities for treating infectious diseases and reducing the drug resistance.⁶⁰ Further studies are needed to explore the molecular basis of the synergistic interaction for developing new natural antibacterial agents.

Table 6. MIC and MBC (mg/mL) of extracts from *P. atlantica*: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions. R = MBC/MIC, bactericidal effect: MBC/MIC $\leq$ 4, bacteriostatic effect: MBC/MIC $>$ 4.

Bacterial strains	CrE			PEE			EAE			AqE		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Ec	–	–	–	–	–	–	0.39	0.39	1.00	–	–	–
Pa	–	–	–	–	–	–	0.39	12.50	32.05	–	–	–
Sa	1.56	0.78	0.50	3.12	3.12	1.00	0.39	0.78	2.00	–	–	–
Bc	0.19	0.39	2.00	1.56	6.25	4.00	0.19	0.39	2.00	–	–	–
St	–	–	–	–	–	–	0.19	12.50	64.10	6.25	25.00	4.00
Ef	–	–	–	–	–	–	3.12	12.50	4.00	–	–	–
Cf	–	–	–	–	–	–	0.19	1.56	8.01	–	–	–
Kp	0.02	0.19	0.81	3.12	0.19	0.06	3.12	12.50	4.00	1.56	1.56	1.00
Pm	6.25	50.00	8.00	–	–	–	0.78	1.56	2.00	–	–	–
Ec 982	–	–	–	–	–	–	3.12	6.25	2.00	–	–	–
Pa 982	–	–	–	–	–	–	0.19	3.12	16.02	–	–	–
Kp 982	–	–	–	–	–	–	3.12	6.25	2.00	–	–	–
Sa 995	1.56	25.00	16.02	1.56	12.5	8.00	0.39	0.78	2.00	–	–	–
Sp 990	–	–	–	–	–	–	0.04	3.12	65.10	–	–	–

Table 7. Antibacterial activity of *P. atlantica* extracts: crude extract (CrE), petroleum ether (PEE), ethyl acetate extract (EAE) and aqueous (AqE) fractions combined with standard antibiotics.

Bacterial strains	Inhibition zone (mm)				
	CrE+AB	PEE+AB	EAE+AB	AqE+AB	AB
<i>Ec</i>	–	–	31.00 (S)	–	28.00 (CTX)
<i>Pa</i>	–	–	17.00 (S)	–	11.00 (CAZ)
<i>Sa</i>	40.00 (S)	40.00 (S)	39.00 (S)	–	27.00 (OXA)
<i>Bc</i>	43.00 (S)	–	–	–	31.50 (IPM)
<i>St</i>	–	–	25.00 (I)	26.00 (I)	25.00 (SXT)
<i>Ef</i>	–	–	26.00 (S)	–	22.50 (AMC)
<i>Cf</i>	–	–	44.00 (S)	–	35.00 (CTX)
<i>Kp</i>	30.00 (S)	28.00 (S)	25.00 (S)	25.00 (S)	20.00 (CTX)
<i>Lm</i>	–	–	–	15.00 (CTX)	–
<i>Pm</i>	33.00 (S)	–	30.00 (S)	–	27.00 (AMC)
<i>Ec 982</i>	–	–	39.00 (S)	–	32.00 (CTX)
<i>Pa 982</i>	–	–	23.00 (I)	–	23.00 (CAZ)
<i>Sa 995</i>	–	26.00 (S)	–	–	22.00 (OXA)

S: synergy, I: indifference, AB: antibiotic

4. Conclusion

In this study, we evaluated the antioxidant and the antibacterial activities of *P. atlantica* extracts. Our findings indicated that the plant extracts possess strong antioxidant properties against several antioxidant systems and demonstrates potent antibacterial effects. The ethyl acetate extract (EAE) showed a powerful capacity in scavenging radical, reducing power and lipid peroxidation. Furthermore, this extract has an interesting antimicrobial effect against a broad spectrum of bacterial strains. These results describe the biological properties of this plant and provide a scientific foundation for its use in Algerian traditional medicine. Further studies are necessary to study the activities of the characterized phytochemicals by HPLC-DAD, which can be useful for therapeutic applications.

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Povzetek

Pistacia atlantica se v tradicionalni medicini v Alžiriji pogosto uporablja za zdravljenje različnih bolezni. Ta študija je bila izvedena z namenom raziskati antioksidativni potencial in protibakterijske lastnosti izvlečkov plodov. Rezultati so pokazali različne količine polifenolov in flavonoidov v različnih izvlečkih. Kvercetin, galna kislina, klorogenska kislina in metil galat so bile prevladujoče spojine v etil acetatnem izvlečku (EAE) in surovem izvlečku (CrE), določene s HPLC-DAD. EAE je bil najučinkovitejši pri lovljenju DPPH in hidroksilnih (OH•) radikalov, vodikovega peroksida (H₂O₂), pri reducirni moči in skupni antioksidativni kapaciteti. Vsi izvlečki imajo sposobnost zaviranja lipidne peroksidacije. Ugotovljen je bil širok spekter protibakterijskih učinkov (10,66 do 29,33 mm). Poleg tega sta časovno odvisen test ubijanja bakterij in razmerje MBC/MIC pokazala, da so bili vsi izvlečki baktericidni proti večini testiranih bakterij, njihova kombinacija z antibiotiki pa je pokazala izrazit sinergistični učinek. Ugotovitve te študije kažejo, da je zdravilna rastlina potencialen vir naravnih antioksidativnih in protibakterijskih spojin, ki bi jih bilo mogoče uporabiti tam, kjer so tovrstne aktivnosti zaželeno.



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