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In vitro antioxidant activities and total phenolic content of extracts from *Pistacia atlantica* desf.

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Abstract:

Pistacia atlantica Desf. belongs to the Anacardiaceae family, endemic in the arid and semi-arid regions of Algeria. The aim of this study was to evaluate the total Phenolic, Flavonoid, Tannin content, and antioxidant activity of extracts of leaves and fruits were measured by 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing power and total antioxidant capacity. The results showed that the best DPPH assay was found in the crude extract and ethyl acetate fraction of leaves (IC_{50} = 0.0273±0.0001 and 0.0419±0.001 mg/ml respectively) more effective than BHA and Ascorbic acid (IC_{50} = 0.08±0.002 and 0.06±0.002 mg/ml respectively). For the total antioxidant capacity, the best activity found in the ethyl acetate fraction of leaves 2319.238±0.58 mM. also the crude extract and butanol fraction of leaves have a better reducing capacity (3652.288±1.91 and 2210.53±0.4 mM respectively). The results suggest that the *P.atlantica* can be used as a potent natural antioxidant.

Key worde: *Pistacia atlantica* Desf**,** Phenolic, Flavonoid, Tannin**,** DPPH scavenging activity**,** ferric reducing power**,** total antioxidant capacity**.**

INTRODUCTION:

Plants are the main source of active natural products which differ widely in terms of structure and biological properties, Its known as secondary metabolites. As known as natural antioxidants. Which are used to protect food from oxidative degradation, pharmaceutical, cosmetic industries and agriculture. Synthetic antioxidants like BHA and BHT have been under reconsideration because of their toxicological effects ricks[1,2]. When the researchers are looking at finding natural antioxidants from plants, which have a good effect than the synthetic. and the researchers explored that antioxidants by play an important role in the prevention of free radical-induced diseases by acting as hydrogen or electron donors, which quench the free radicals and converted to antioxidant radicals[3]. In recent years, the prevention of cancer and cardiovascular diseases have been associated with the ingestion of fresh fruits, vegetables or plants rich in natural antioxidants[4].

The genus *Pistacia* belongs to the family Anacardiaceae, consisting of 11 or more species, which are shrubs or trees[5,6], it is widely species distributed in the Mediterranean and middle east areas, several endemic species of *Pistacia* are native and common of Algeria territory (*P.lentiscus*, *P.therebinthus* and *P.atlantica*)[7], *P.atlantica* Desf. Subsp *atlantica* is a tree which can reach over 15 m in height and grows in arid and semi-arid areas of Algeria[8]. Its vernacular name is "Butom"[9].

Pistacia species are known for their potent antioxidant properties and also for their antimicrobial, anti-inflammatory and cytotoxic activities[10], different parts of *P.atlantica* Desf. including the resin, leaves and fruits have been widely used as traditional medicine for the treatment of various conditions such as gastrointestinal, respiratory, coetaneous, renal and infectious diseases, pain, peptic ulcer[11,12], Moreover, previous studies have indicated the anti-inflammatory, antioxidant, anti-tumour, anti-asthmatic and antimicrobial properties of these plants[13]. The aim of this study was carried out in order to evaluate the total polyphenols contents and the antioxidant activity of leaves and fruits of *P.atlantica* Desf.

MATERIALS AND METHODS:

Plant material:

The parts of *P.atlantica* Desf. (leaves and fruits) were collected in the month of April-October 2017 in the area of Messed – Djelfa, in the south of Algeria. The plant material was identified by Halis Youcef researcher in Touggourt's scientific and technical research centre for Arid Areas.

Preparation of the extract and fractions:

The leaves and fruits of *P.atlantica* (20 g) were macerated at room temperature with 70% (v/v) aqueous methanol for 48 h, Three times. After filtration; the filtrate was evaporated. Recovered with distilled water and partitioned successively using Chloroform, Ethyl acetate, and n-butanol. The extracts were concentrated and re-dissolved and kept at 6 C°. We have obtained: crude extract, chloroform fraction ($CHCl_3$), ethyl acetate fraction (EtOAc) and butanol fraction (BuOH).

Total Phenolic Contents (TPC):

Total Phenolic Contents (TPC) in the extracts of *P.atlantica* Desf. was determined by the spectroscopic method using Folin-ciocalteu reagent[14]. Briefly; 0.1 ml of the extract was mixed with 0.5 ml of Folin-ciocalteu reagent (10%). After 5 min added 2 ml of (20%) sodium carbonate. After 30 min incubation at room temperature. The absorbance was measured at 760 nm. The TPC was expressed as mg Gallic acid equivalents per gram of dry matter (mg GQE/ g DM).

Total Flavonoid Contents (TFC):

Total Flavonoid Contents (TFC) in the extracts of *P.atlantica* Desf. was estimated by the colourimetric method using aluminium chloride[15]. Briefly, 1.5 ml of 2 % of $AlCl_3$ ethanol solution was added to 1.5 ml of extract. After 30 min incubation at room temperature. The absorbance was measured at 430 nm and the results were expressed as mg Quercetin equivalents per gram of dry matter (mg QE/ g DM).

Total Tannin Contents (TTC):

Total Tannin Contents (TTC) was estimated by using the vanillin assay method.[16] 3 ml of 4 % ethanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added to 0.5 ml of extract and allowed to react at room temperature for 15 min. the absorbance was measured at 500 nm. the TTC was calculated as mg of Catechin equivalent per gram of dry matter (mg CE/ g DM).

DPPH Radical Scavenging Activity:

Radical scavenging activity of *P.atlantica* Desf. extracts were determined by using DPPH assay [17]. Briefly, 0.1 ml of various concentration of extracts were mixed with 1.9 ml of 0.1 mM DPPH ethanol solution. Finally, the solutions were incubated for 30 min in the dark at room temperature. The reaction of the DPPH radical was estimated by measuring the absorption at 517 nm and ascorbic acid was used as a positive control. Inhibition of DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect } (\%) = \frac{A0 - A1}{A0} \times 100$$

Where A0 and A1 are the absorbances 30 min of the control and the sample respectively.

Total antioxidant capacity:

The total antioxidant capacity of the different extracts of *P.atlantica* Desf. was determined according to the method phosphomolybedenum assay[18,19]. 0.3 ml of different concentration of the extracts were mixed with 3 ml of standard reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammoniummolybdate). Then, the reaction mixture was incubated at 95 C° for 90 min. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm, using ascorbic acid as a positive control and the results were expressed as mM equivalent ascorbic acid.

Reducing power assay (FRAP):

The reducing power of *P.atlantica* Desf. extracts were determined according to the method of Oyaizu[20]. Some modification, 0.2 ml of different concentration of the extract were mixed with 0.5 ml of phosphate buffer (pH 6.6) and 0.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 C° for 20 min. After incubation, 0.5 ml of Trichloracetic acid (TCA 10 %), After 10 min, 1.7 ml of the mixtur**e** was mixed with 1.7 ml distilled water and 0.34 ml of Ferric Chloride (0.1%) were added to it. the absorbance was read at 700 nm, using ascorbic acid as a positive control and the results were expressed as mM equivalent ascorbic acid.

Statistical analysis: Data analyses were expressed as means ± standard derivation (SD) using Excel programmer. All experiences were repeated in triplicates.

RESULTS AND DISCUSSION:

Total Phenolic, Flavonoid and Tannin Contents:

The TPC of the leaves and fruits of *P.atlantica* were measured using the folin-ciocalteu method. The results were shown in table.1. The values varied from 0.088 ± 00 to 12.97 ± 0.03 mg GAE/ g DM, the highest TPC was observed in the crude extract of fruits and n-butanol fraction (leaves 5± 0.0225 and fruits 6.389± 0.3 mg GAE/ g DM) while the lowest was in the Chloroform fraction of fruits 0.088±0.00, followed by the Chloroform fraction of leaves 0.606±0.008 mg GAE/ g DM. TFC and TTC of the plant extracts were also measured (table 1), for TFC the values ranged from 0.006±0.00 mg QE/ g DM for Chloroform fraction of fruits to 1.388±0.0069 mg QE/ g DM for a crude extract of leaves. Whereas the levels of TTC varied from 0.00389±0.001 to 0.613±0.04 mg CE/ g DM.

Table1: Total Phenolic, Flavonoid and tannin contents.

Extract		Total phenolics (mg GAE/g DM)*	Total flavonoids (mg QE/g DM)*	Total tannins (mg CE/g DM)*
Crude extract	Leaves	4.82 ± 0.099	1.388 ± 0.0069	0.613 ± 0.04
	Fruits	12.97 ± 0.3	0.825 ± 0.007	0.118 ± 0.001
Chloroform fraction	Leaves	0.606 ± 0.008	0.0407± 0.00	0.201 ± 0.001
	Fruits	0.088 ± 0.00	00.006 ± 0.0	0.00389 ± 0.001
Ethyl acetate fraction	Leaves	1.765 ± 0.017	0.158 ± 0.0025	0.0723 ± 0.0006
	Fruits	1.265 ± 0.02	0.086 ± 0.00	0.0059 ± 0.002
n-butanol fraction	Leaves	5 ± 0.0225	1.318± 0.0037	0.177 ± 0.002
	Fruits	6.389± 0.3	0.362 ± 0.006	0.024 ± 0.001

** Results are expressed as the mean of 3 values ± standard deviation*

Table 2: DPPH Scavenging, Total antioxidant activity and Reducing power.

Extract		DPPH IC50 (mg/ml)*	Molybdate (mM)*	FRAP (mM)*
Crude extract	Leaves	0.0237± 0.0001	2226.179± 0.5	3652.285 ± 1.91
	Fruits	1.252± 0.006	21.572± 0.05	48.6± 0.5
Chloroform fraction	Leaves	0.536±0.019	1348.327± 2.48	578.501±0.77
	Fruits	1.016 ± 0.007	302.571± 0.2	265.532± 0.5
Ethyl acetate fraction	Leaves	0.0419±0.001	2319.238 ± 0.58	1797.813±2.55
	Fruits	0.089±0.002	722.88± 2.37	1908.757± 0.64
n-butanol fraction	Leaves	0.046 ± 0.001	1683.06± 1.2	2210.53± 0.4
	Fruits	0.258±0.002	115.60± 0.2	209.3± 0.46
Ascorbic acid	0.06 ±0.002		/	/
BHA	0.08 ±0.002		7.17±0.05	10.74± 0.04
BHT	/		14.676±0.01	07.41± 0.02

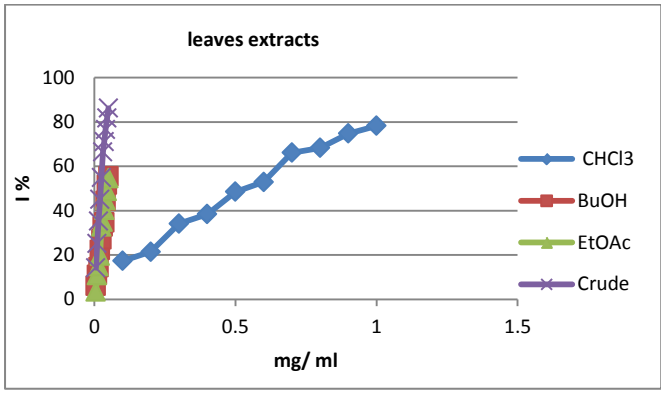
** Results are expressed as the mean of 3 values ± standard deviation*

DPPH Radical Scavenging Activity:

The free radical scavenging activity of extracts from leaves and fruits of *P.atlantica* Desf. along with the reference standard ascorbic acid and BHA were determined by the DPPH method as shown in table 2. The method is based on the reduction of the stable free radical DPPH with violet colour by donating hydrogen from the Phenolic hydroxyl groups to non-radical DPPH-H with a yellow colour.

DPPH values of IC_{50} ranged between 0.0237±0.001 to 1.252±0.006 mg/ml, the best activity was observed in the crude extract and ethyl acetate fraction, butanol fraction of leaves 0.0237±0.001 and 0.0419±0.001, 0.046±0.001 mg/ml respectively. Which are better than the reference antioxidant ascorbic acid 0.06±0.002 and BHA 0.08±0.002 mg/ml.

The lowest scavenging activity was recorded in the crude extract and $CHCl_3$ fraction of fruits 1.252±0.006 and 1.016±0.007 mg/ml, and IC_{50} the ethyl acetate fraction of fruits 0.089±0.002 had near the activity of the BHA 0.08±0.002 mg/ml.



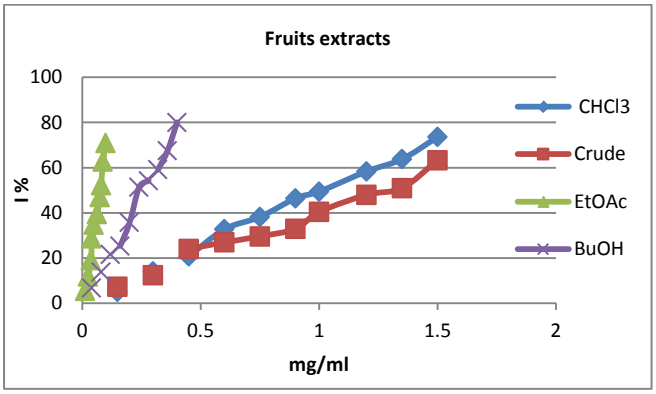


Fig1. DPPH radical scavenging activity of leaves and fruits extracts of *P.atlantica* Desf.

Total antioxidant capacity:

The total antioxidant activity of the extracts of leaves and fruits of *P.atlantica* Desf. was measured by the phosphormolybdenum method, which is based on the reduction of Mo (VI) to Mo (V). the formation of green phosphate Mo (V) compounds[21].

The results are summarized in table 2.the values of total antioxidant varied between 2319.2383±0.58 and 21.572±0.05 mM, the leaves extracts of *P.atlantica* gave the higher result of reduction of Mo (VI) exactly in the ethyl acetate fraction 2319.2393±0.58 followed by the crude extract 2226.1787±0.5 mM, while the lowest was recorded in the extracts of the fruit (crude extract 21.572±0.05 and butanol fraction 115.60±0.2 mM).

All the extracts showed a strong antioxidant activity better than BHA 7.17±0.05 and BHT 14.676±0.01 mM (fig.2).

Reducing power assay:

The reduction capacity of a compound may serve as a significant indicator of this potent antioxidant activity[22]. Antioxidant potential of extracts from *P.atlantica* Desf. was estimated by to reduce ferric iron Fe^{3+} to the ferrous Fe^{2+} was recorded by measuring the formation of Perl's Prussian blue at 700 nm, thus measuring the ability of extracts to interact with the reactive species such as free radicals as an electron donor[23] . Fig.3 shows the reducing power activities of leaves and fruits extracts of *P.atlantica* Desf.

Ferric reducing activity ranged from 3652.285±1.91 to 48.6±0.5 mM. the crude extract and butanol fraction of leaves had the best reducing activity with a values 3652.285±1.91 and 2210.53±0.4 mM, followed by the ethyl acetate fraction from fruits 1908.757±0.64 and 1797.813±2.55 mM for ethyl acetate fraction of leaves. the lowest reducing activity was recorded in crude extract and butanol fraction of fruits (48.6±0.5 and 209.3±0.46 respectively). The chloroform fraction of leaves and fruits also have a good ferric reducing activity among 578.50±0.77 and 265.532±0.5 mM respectively. And all extracts showed excellent reducing activity better than synthesis antioxidants BHA 10.74±0.04 and BHT 7.41±0.02 mM.

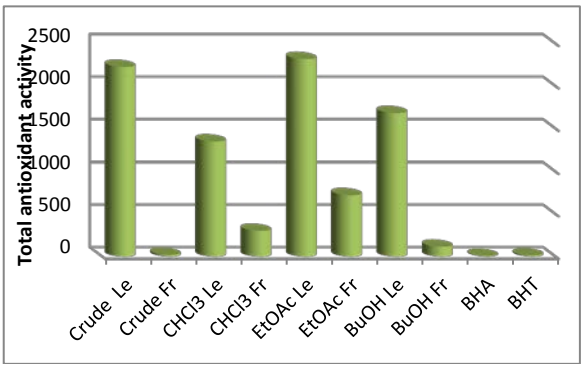


Fig.2 Total antioxidants capacity of leaves and fruits extracts of *P.atlantica* Desf. (Le: leaves; fr: fruits)

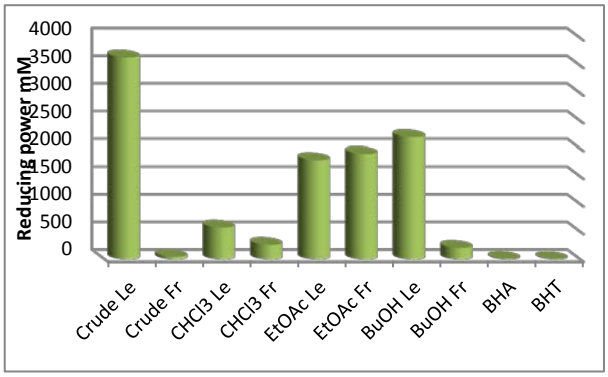


Fig.3 Reducing power activities of leaves and fruits extracts of *P.atlantica* Desf. (Le: leaves; fr: fruits)

CONCLUSIONS:

On the basis of the results of this study, the *P.atlantica* Desf had the bioactive compounds (Phenolic, Flavonoid and tannin content) which exhibited higher antioxidant activities, the ethyl acetate and butanol fraction showed the better results in all the in vitro models of antioxidant assays studied, also the crude extract of leaves. This results may confirm the traditional use of the plant, for that further work which aims the determination of antioxidant activity in vivo, anti-inflammatory, anticancer activities and advanced fractionations of the different extracts of *P.atlantica* Desf.

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