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IN-VITRO ANTIOXIDANT EFFECTS OF TANNIN EXTRACTS OF *PISTACIA ATLANTICA*

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ABSTRACT: Seven parts of *Pistacia atlantica* were collected from Tlemcen in Algeria (fruits, leaves, buds, stems, roots internal and external trunk barks). Tannin content was determined and antioxidant activity of tannic extracts were measured using different assays: Total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power (FRAP) and β-carotene bleaching assay The results showed that the highest tannin contents were observed in roots(49.789±0.391 mg CE/g DM) and internal trunk barks (49.095±0.306 mg CE/g DM). Tannins extracted from leaves, roots and external trunk barks showed very good DPPH radical scavenging activity (IC50 of 0.124±0.001, 0.304±0.001 and 0.440±0.001 mg/mL), ferric reducing power (0.021±0.001, 0.154±0.001 and 0.170±0.005 mg/mL) and β-carotene bleaching inhibition (0.313±0.009, 0.094±0.006 and 0.131±0.002 mg/mL), respectively. Therefore, the results suggest that this plant with all its studied parts might be considered as a potential candidate of possible health-promoting functional foods.

INTRODUCTION: *Pistacia atlantica* Desf., is a tree located in north Africa, which can reach over 15 m in height and grows in arid and semi-arid areas, vernacularly called "Butom", valued in Algeria because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases [1], also used for stress, as tonic, and antidiarrheal [2].

Moreover, *P. atlantica* has long been used in folk for different medicinal purposes such as mouth flavouring, tanning, and as fodder in Greece [3], for mouth disease and wound healing in Turkey [4], stomach ache and as an antidiabetic in Jordan [5], as mouth freshener, antiseptic, gum tissue strengthener and antidiarrheal in Iran [3], as gum tissue strengthener, for breath deodorizer, cough, chill, and stomach disease in south Africa [6].

Tannins, the non-nutritive substances from plant sources exhibit potent biological activities that lower the risk of chronic diseases without any side effects. Tannins are most abundant and multipotent molecules exhibiting antioxidant, proapoptotic and antiangiogenic activity and thus being effective in the treatment of degenerative diseases including initiation and progression of tumors [7].

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There have been few studies on tannic compounds in leaves and fruits of *P.atlantica*, which are potent antioxidants and free radical scavengers [8, 9, 10, 11, 12]. According to our knowledge, there is no study reported the antioxidant activity andtanninc profile of all parts of *P.atlantica.*

Due to lack of adequate knowledge about antioxidant activity of different parts of *P.atlantica*, this study was carried out in order to evaluate tannic profile of the different extracts and their antioxidant activity was further evaluated by different assays.

MATERIAL AND METHODS:

Standards and reagents:

All standards and chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Plant material:

The different parts of *P. atlantica* Desf. (fruits, leaves, buds, stems, trunk barks and roots) were collected from Abu Tachfine, Tlemcen, in Algeria, at February-August 2011. Trunk barks were divided to internal and external barks, to be investigated separately. The samples were air-dried in shadow at room temperature, and reduced to fine powder, just before extraction.

Preparation of methanolic extracts:

A fine dried powder of samples (2 g) was extracted using 60mL of methanol for 24 h at room temperature. After filtration through filter paper, the resulting solution was evaporated under vacuum at 60°CbyBuchi RotavaporR-200dry. The residue was then dissolved in 3mL of methanol [13].

Extraction of tannins:

Tannins extracts were prepared as described by [14]. The powder (2.5 g) of dry and powdered samples was extracted with 100mL of acetone-water (70/30, v/v), and the mixture was stirred continuously for 72 h, at room temperature. The mixture was then filtered and evaporated under vacuum at 40°C to remove acetone. The remaining solution was washed twice with 30mL of dichloromethane to remove lipid soluble substances. After the elimination of dichloromethane under reduced pressure, the aqueous phase was also extracted twice with 30mL of ethyl acetate. Finally, the organic phases of ethyl acetate were recovered and evaporated to dryness. The obtained residues were weighed and well preserved to be used later.

Condensed tannin content:

Tannin content was estimated using the vanillin assay method [15]. Fifty microliters (50µl) of each methanolic extract was added to 1500µl of vanillin/methanol solution (4%, w/v) and vortexed. Then, concentrated HCl (750µl) was added and allowed to react at room temperature for 20 min. The absorbance at 550 nm was then measured against a blank. The concentration of tannins was calculated as milligrams of catechin equivalents per gram of dry matter (mg CE/g DM) from a calibration curve. All the tests were carried out in triplicate.

Total antioxidant capacity:

The total antioxidant capacity of tannin extracts was determined according to the method of[16]. Briefly, 0.3 ml of sample was mixed with 3 ml of standard reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammoniummolybdate). Then, reaction mixture was incubated at 95 °C for 90 min. After, the mixture had cooled to room temperature and the absorbance was measured at 695 nm. Total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of dry matter–(mg AAE/g DM) and as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). The sample was analyzed in triplicate.

DPPH radical scavenging activity assay:

DPPH radical scavenging activity assay was performed as described by Sanchez-Moreno. (1998) [17]. Fifty microliters of various concentrations of tannins (0.0625–1 mg/mL) was mixed with 1950 µL of a methanolic solution containing 0.025 g/L DPPH (2,2-diphenyl-1-picrylhydrazil). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The reaction of the DPPH radical was estimated by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$RSA\ (\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound); A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotted of inhibition percentages against extract concentrations. BHA was used as the reference compound.

Reducing power assay:

The reducing power was determined as described by (Oyaizu, 1986) [18]. A volume of 1 mL of various concentrations of tannins (0.0125-0.2 mg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH=6.6) and 2.5 mL of 1% of potassium ferricyanide water solution ($K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. A volume of 2.5 mL of trichloracetic acid (10% aqueous solution) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. BHA was used as standard reference. In this assay, the higher absorbance indicates the higher reducing power. EC_{50} value (mg/ml) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

β-carotene bleaching assay:

The antioxidant activity of tanninextractswas evaluated using β-carotene-linoleate model system, as described by Moure et al. (2000) [19]. β-carotene (2 mg) was dissolved in 10 ml chloroform and 1 ml β-carotene solution was mixed with 20 µL of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was then evaporated under vacuum evaporator and the resulting mixture was immediately diluted with 100 ml of distilled water. To an aliquot of 4 ml of this emulsion, 0.2 ml of various concentrations extracts (0.125–2 mg/ml) or the reference antioxidant (BHA) was added and mixed well.

The absorbance at 470nm was immediately recorded after adding the sample to the emulsion, which was regarded as t = 0 min, against a blank consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50 °C for 120 min. A negative control consisted of 0.2 mL distilled water or solvent instead of extract or reference antioxidants. The antioxidant activity (AA) was calculated to the following equation:

$$AA= ((A_{A(120)} - C_{C(120)} / (C_{C(0)} - C_{C(120)}) \times 100$$

Where $A_{A(120)}$ is the absorbance of the antioxidant at120 min;$C_{C(120)}$ is the absorbanceof the controlat 120min;$C_{C(0)}$ is theabsorbanceof the controlat 0 min.

Statistical analysis:

Data analyses were expressed as means ± standard derivation (SD) using Excel programme and Microcal Origin 6. For the quantification of condensed tannins and total antioxidant capacity, all experiences were repeated in triplicates. All evaluations of antioxidant activity were performed in two replicates.

RESULTS AND DISCUSSION:

Determination of condensed tannin content:

The quantification of condensed tannins of seven parts of *P. atlantica*. Furthermore is shown in **Table 1.** Among parts analyzed here, roots contained the highest amount of tannin (49.789±0.391 mg CE/g DM), followed by internal trunk barks (49.095±0.306 mg CE/g DM) and buds (48.615±0.141 mg CE/g DM). The contents in roots, internal trunk barks and buds were respectively 49.789±0.391, 49.095±0.306 and 48.615±0.141 mg CE/g DM,. For the other parts, the range of contents varied between 5.178±0.126and 24.653±0.513mg CE/g DM.

Our results found in this investigation were different compared to those reported by Belyagoubi-Benhammou et al., (2014) [10] as 3.06±0.15CE/g DM. That may be due to both genetic and environmental factors that influence plant phenolic composition [20].

Total antioxidant capacity:

The total antioxidant capacity of the tannins was expressed as the number of equivalents of ascorbic and gallic acids **(Table 2)**. Tannic extracts showed higher values and indicated higher total antioxidant

capacity. Leaves (53.204±0.079 mg GAE/g DM; 33.437±0.049 mg AAE/g DM) showed the highest antioxidant effect, followed by buds, stems, fruits, internal trunk barks, roots and finally external trunk barks with the lowest antioxidant capacity (5.061±0.488 mg GAE/g DM; 3.181±0.306mg AAE/g DM).

The results indicate that the antioxidant capacity of tannic extracts seems to be due to the nature and not to the concentration of tannins, that may be acted upon by donating electrons to the free radicals and the neutralize them all.

TABLE 1: TANNIN CONTENTS IN SEVEN PARTS OF *P.ATLANTICA*.

Plant parts	Tannin content (mg CE/g DM)
Fruits	5.178±0.126
Leaves	11.805±0.307
Buds	48.615±0.141
Stems	24.653±0.513
Internal trunk barks	49.095±0.306
External trunk barks	5.913±0.031
Roots	49.789±0.391

Values were the mean of three replicates ± SD; DM: dry matter; CE: catechin equivalents.

TABLE 2: TOTAL ANTIOXIDANT CAPACITY, EC_{50}OF DPPH RADICAL SCAVENGING, REDUCING POWER AND β-CAROTENE BLEACHING ASSAYS OF TANNINS OF SEVEN PARTS OF *P.ATLANTICA*.

Plant parts	Total antioxidant capacity		EC_{50} (mg/mL)		
	mg AAE/g DM	mg GAE/g DM	DPPH assay	Reducing power	β-carotene assay
Fruits	24.318±0.036	15.283±0.022	0.164±0.001	0.127±0.001	0.415±0.020
Leaves	53.204±0.079	33.437±0.049	0.124±0.001	0.021±0.001	0.313±0.009
Buds	36.197±0.624	22.749±0.392	0.143±0.005	0.245±0.006	1.624±0.004
Stems	31.013±0.231	19.491±0.145	0.208±0.009	0.086±0.001	4.190±0.534
Internal trunk barks	11.160±0.204	7.014±0.128	0.234±0.003	0.171±0.002	2.136±0.309
External trunk barks	5.061±0.488	3.181±0.306	0.440±0.001	0.170±0.005	0.131±0.002
Roots	10.541±0.326	6.624±0.205	0.304±0.001	0.154±0.001	0.094±0.006
BHA			0.090±0.001	0.060±0.001	0.243±0.002

Values were the mean of two replicates ± SD. DM: dry matter; GAE: gallic acid equivalents; AAE: ascorbic acid equivalents

DPPH assay:

The free radical-scavenging activity of tannins from different parts of *P.atlantica* along with the reference standard BHA were determined by the DPPH assay as shown in **Table 2**. In accordance with findings from total antioxidant capacity assay, DPPH values of EC_{50}were ranged from 0.164±0.001mg/mL for leaves, 0.124±0.001mg/mL for buds and 0.143±0.005mg/mL for fruits to 0.440±0.001 mg/mL for external trunk barks. The radical scavenging power of all tannin extracts was less than that of BHA (0.090±0.001 mg/mL).

The results presented above showed that EC_{50} in DPPH assay decreases in a concentration-dependent manner of remaining DPPH indicated that *P.atlantica* possesses potent free radical-scavenging activity.

Reducing power assay:

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity [21]. Antioxidant potential of tannins from *P.atlantica* was estimated from their ability to reduce ferric ion Fe^{+3} to ferrous Fe^{+2}. In brief, the reducing power of tannins followed the order: Leaves 0.021±0.001 mg/mL better than that of BHA (0.060±0.001mg/mL) stems and fruits came after with 0.086±0.001 and 0.127±0.001 mg/mL, respectively **(Table 2)**. EC_{50} of remaining extracts were between 0.154±0.001 and 0.245±0.006 mg/mL. However, FRAP assay showed that different parts of *P.atlantica* with potent activity can be introduced as a natural source of antioxidants.

β-carotene bleaching assay: In the β-caroten/linoleic acid method, β-carotene undergoes

rapid discoloration in the absence of an antioxidant. The presence of an antioxidant can hinder the extent of β-carotene destruction by neutralizing the linoleate free radical and any other free radicals formed within the system [22]. In this test, the most potent part was roots (EC_{50}=0.094±0.006 mg/mL) followed by one the smaller tannin concentrations, which is the external trunk barks indicates the second higher activity (EC_{50}=0.131±0.002 mg/mL) even more active than that of BHA (0.243±0.002 mg/mL) **(Table 2)**. The other tannic extracts were between 0.313±0.009 mg/mL for leaves and 4.190±0.534 mg/mL for stems. Finally, the test of β-carotene reveals the very promising potent activity of these extracts of *P. atlantica* as a source of natural antioxidants.

CONCLUSION: The present study reveals that *P.atlantica* had a relatively high level of extractable condensed tannins. Furthermore, as far as our literature review is concerned, this research is the first study which reports results of antioxidant activities and tannic profile of all parts of *P.atlantica*.

Tannins extracted from leaves, roots and external trunk barks showed very good total antioxidant capacity, DPPH radical scavenging activity, ferric reducing power and β-carotene bleaching inhibition. It had been reported that the antioxidant activity of plant extract is correlated with the amount of their phenolic compounds [23].

However, roots, internal trunk barks and buds with high levels of tannic compounds can be used as potent natural antioxidants in food and pharmaceutical industries and may be useful in preventing or reducing the progress of diseases linked to free radical overproduction, such as cardiovascular diseases and cancer.

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