



Research article

Assessment of aridity effects on phytochemistry and ecophysiology of *Argania spinosa* (L.)

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Abstract

Argania spinosa is a species that grows in arid and semi-arid areas of Morocco. The phytochemistry and ecophysiology of argan leaves from seven localities; Essaouira, Tamanar, Imouzzar, Admine, Aït Baha, Merghat and Bouizakarne were studied to investigate the different factors engaged in the resistance of this species to the environmental conditions in these areas. The relative water content in the argan tree leaves of Bouizakarne and Ait Baha was lower with a value of 55.09% and 63.9% successively compared to the other areas and there was an increase in the content of solutes (soluble sugars and prolin), proteins with 505.21mg/g DW in leaves of Bouizakarne and 327.1 mg/g DW in those of Ait Baha and total phenols (8.76 mg/g DW and 8.1 mg/g DW successively), in particular the flavonoids, and there were substantial amounts in the tree leaves of Bouizakarne and Ait Baha. Based on high-performance liquid chromatography, there were no qualitative differences in the argan leaf extracts concerning flavonoids among the seven studied areas. Furthermore, there was no significant difference between these seven localities regarding the antioxidant activity, the chlorophyll pigments contents a and b and the carotenoids.

Introduction

Argan, *Argania spinosa* (L.) Skeels is a multipurpose tree that is endemic to south-western Morocco, which currently contains arid and semi-arid areas. Unfortunately, the area occupied by this species has declined due to the large amount of clearing related to anthropogenic direct action, over-shepherding and deforestation, resulting in forest degradation (Guinda et al., 2011).

Species in the arid and semi-arid areas can survive a water deficit through a set of mechanisms. The relative water content and water potential are the most studied parameters to evaluate the plant water state (Tezara et al., 2002).

Water stress causes the establishment of a plant's water regulatory state which is manifested by the accumulation of osmoregulatory compounds leading to osmotic potential reduction, so allowing the maintenance of turgor potential. Sugars and free amino acids are the most important solutes accumulating in plants under water deficit conditions (Arve et al., 2011).

Water stress affects protein biosynthesis, by increasing their content. Indeed, the proteins are considered as a biochemical criterion in plant reactions when subjected to environmental constraints (Bacelar et al., 2006)

Phenolic compounds, especially flavonoids, are secondary metabolites used in the chemotaxonomy of the principal plant groups (Mitra et al., 2012). They are involved in a large number of physiological processes in plants acting as stress biomarkers to ensure plant survival under different environmental conditions

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(Mitra et al., 2012). Several studies have demonstrated the role played by flavonoids in this way (Diabate et al., 2009) especially in the argan tree (Tahrouch, 2000), whose content of these compounds is noteworthy and can be affected by weather conditions and other environmental factors. These metabolites are also natural antioxidants that are arousing more and more interest for cancer prevention and treatment and to address inflammatory and cardiovascular disease (da Costa et al., 2015). Recently, there has been increased interest in natural antioxidants, regarding their therapeutic properties. These substances have phenolic hydroxyl groups in their structures and the antioxidant properties are attributed in part to the ability of these natural compounds to scavenge free radicals (Popovici et al., 2009).

In this study, we have compared phytochemical and ecophysiological characteristics of the argan trees derived from seven localities in southern Morocco, according to an increasing aridity gradient, to study the behavior of this tree against the aridity.

Materials and Methods

Plant material

The plant material was collected from seven localities: Essaouira, Tamanar, Imouzzar Ida Outanane, Admine, Ait Baha, Merght and Bouizakarne. The material consisted of argan leaves taken from 15 different trees in each locality (Table 1).

A part of the samples collected was dried in an oven at 40°C for 48 hr and then reduced to a fine powder. The other part was retained in the freezer for analysis.

Relative water content

The relative water content (RWC) of leaves was evaluated according to Equation 1 (Whetherley, 1950):

$$RWC (\%) = (FW - DW) / (TW - DW) \times 100 \quad (1)$$

where: FW is the fresh weight, TW is the turgid weight estimated after soaking the leaves in distilled water in the dark for 24 hr at 4°C and DW is the dry weight measured after oven-drying the leaves for 48 hr at 70°C.

Determination of photosynthetic pigments

A fresh leaf sample (0.5 g) was mixed with 50 mL of 90% acetone and then centrifuged at 3,500×g for 10 min. Quantification was performed spectrophotometrically by measuring the absorbance of extract at 663, 648 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The pigment concentrations were then calculated using Equations 2–4 (Lichtenthaler, 1987):

$$\text{Chlorophyll } a = (11.75 * A_{663} - 2.35 * A_{648}) \quad (2)$$

$$\text{Chlorophyll } b = (18.61 * A_{648} - 3.96 * A_{663}) * 50/500 \quad (3)$$

$$\text{Carotenoids} = ((1,000 * A_{470}) - (2.27 * chl\ a) - (81.4 * chl\ b)) / 227 * 50/500 \quad (4)$$

where A663, A648 and A470 are the absorbance of extract at 663, 648 and 470 nm, respectively, and chlorophyll a (chl a) and b (chl b) and carotenoid contents were all measured in milligrams per gram fresh weight.

Prolin content determination

The content of prolin was measured using the method of Paquin and Lechasseur (1979). A sample (5 mL) of 95% ethanol volume per volume (v/v) was added to 0.4 g of dried tissue powder. After homogenization, the mixture was centrifuged at 3,500×g for 10 min and the supernatant was recovered. The extraction procedure was repeated three times by washing the pellet with 5 mL of 70% ethanol. A 5 mL aliquot of pooled supernatants was incubated at 0°C for 12h in

Table 1 Geographic and climatic data of argan tree sampling

Locality	Position	Geographic origin	Altitude (m)	Annual rainfall (mm)	Climate
Essaouira	N31°05'14.5'' W009°40'32.4''	Atlantic coast	117	280	Semi-arid
Tamanar	N30°59'30'' W009°42'42.9''	Atlantic coast	183	300	Semi-arid
Imouzzar Ida Outanane	N30°40'23'' W009°28'48''	Northern side of the Anti-Atlas	900	120–422	Semi-arid
Admine	N30°20'3'' W 9°27'50''	Souss plain	90	239	Arid
Ait Baha	N30°03'49.7'' W009°07'15.3''	Northern side of the Anti-Atlas	610	120	Arid
Merght	N29°25'18.5'' W009°42'29''	Southern side of the Anti-Atlas	712	250–300	Arid
Bouizakarne	N29°09'57.7'' W009°43'34.1''	Northern limit of the Sahara	629	146	Arid

the presence of 2 mL chloroform and 3 mL distilled water. Thereafter, 1 mL of the upper phase of the extract was diluted with 10 mL of distilled water and mixed with 5 mL of ninhydrin (0.125 g ninhydrin, 2 mL of 6M orthophosphoric acid and 3 mL of glacial acetic acid) and 5 mL of glacial acetic acid. The reaction mixture was incubated at 100°C for 45 min and then cooled. The chromophore was extracted with 4 mL toluene and its absorbance was measured at 515 nm using an IC 6400 visible spectrophotometer. The prolin concentration in milligrams per gram dry weight (DW) was determined using a standard curve.

Determination of soluble sugar content

The soluble sugars content was determined according to the method of Dubois et al. (1956). An amount of approximately 0.02 g of dry powdered leaves was mixed with 2 mL of 70% ethanol (v/v) and then centrifuged at 2000×g for 10 min. The supernatant was recovered and the extraction was repeated two times on the pellet. The supernatants were combined and 16 mL of distilled water were added (Sánchez et al., 1998). Afterward, 200 µL of the extract was treated with 200 µL of 5% phenol and 1 mL of concentrated sulfuric acid (Dubois et al., 1956). After 20 min of incubation at 30°C, the absorbance was measured at 490 nm using an IC 6400 visible spectrophotometer. The contents of soluble sugar were determined using glucose standard and expressed as milligrams per gram DW.

Determination of total protein content

Quantification of total soluble protein was carried out using the method described by Lowry et al. (1951). Thus, a quantity of 0.1 g of dried leaves powder was homogenized with 1 mL of lysis buffer (8 mL 1M Tris-HCl pH 6.8, 2 mL β-mercaptoethanol, 10 mL SDS and 80 mL water) and the mixture was centrifuged 10 min at 13,000×g. Then, 10 µL of supernatant was mixed with 990 µL of water and 5 mL of Lowry solution. The reaction mixture was incubated in darkness at room temperature for 10 min, and then 0.5 mL of 50% Folin-Ciocalteu reagent was added to it. Thereafter, the absorbance was measured at 750 nm using an IC 6400 visible spectrophotometer. The protein content in milligrams per gram DW was determined using bovine serum albumin as standard.

Determination of total phenolics

To extract the total phenolic compounds, an amount of 50 mg of powdered plant material was soaked in 2.5 mL of 95% ethanol and kept in the freezer for 48 hr. After homogenization, the mixture was centrifuged for 10 min at 13,000×g. An aliquot (1 mL) of supernatant was homogenized with 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. The mixture was left to stand for 5 min, and then 1 mL of 5% Na₂CO₃ was added. After agitation, the reaction mixture was kept in the dark for 1 hr and the optical density was measured at 725 nm using the IC 6400 visible spectrophotometer (Ben Dkhil and Denden, 2010). The phenolic content was reported milligrams per gram DW using gallic acid as the standard.

Determination of total flavonoids

A sample (50 mg) of dry powdered leaves was placed in an Eppendorff tube and homogenized with 1 mL of 80% methanol. The mixture was sonicated for 15 min and then centrifuged at 3,500×g for 30 min. The obtained extracts were used for total flavonoids determination, antioxidant activity and high-performance liquid chromatography.

The total flavonoids quantification was done using a spectrophotometer and two different methods. In the first method, the flavonoids concentration was determined by calibration range. Different concentrations of quercetin (0.05 mg/mL) were prepared and the absorbance was determined at 350 nm.

The second method used was the Andary method (Andary, 1990). A sample (2 mL) of the extract was mixed with 100 µL of Neu's reagent (Neu, 1956; 1% methanolic solution of diphenylboric acid-2-aminoethyl). The sample absorbance was determined at 404 nm. The total amount of flavonoids was calculated using Equation 5 (Hariri et al. 1991):

$$F = A_{ext} * 0.05 * 100 / A_q * C_{ext} \quad (5)$$

where A_{ext} is the extract absorbance, A_q is the quercetin absorbance (0.05 mg/mL) and C_{ext} is the concentration of extracted plant material measured in milligrams per milliliter.

The flavonoid concentration was reported as milligram equivalents quercetin per gram DW.

Antioxidant activity

The DPPH scavenging method was used to evaluate the antioxidant potential of extracts. An aliquot of 50 µL of extract was added to 950 µL of the methanolic solution of DPPH (0.1 mM). After 30 min, the absorbance was measured at 517 nm against a blank which corresponded to DPPH solution without extract. The percentage inhibition of free radical DPPH was calculated according Equation 6 (Loo et al., 2008):

$$P = (A_1 - A_2) * 100 / A_1 \quad (6)$$

where P is the percentage of DPPH radical scavenger, A_1 is the absorbance of the control reaction (DPPH solution without extract) and A_2 is the absorbance in the presence of the sample.

High-performance liquid chromatography

HPLC analysis was performed using the isocratic mobile phase, consisting of a ratio of acetonitrile to MeOH to H₂O of 2:8:15. The chromatography was carried out on a Nucleosil C18 column (internal diameter 4 mm, height 250 mm and particle size 5 mm). Ultra violet-visible data were recorded using a photodiode array detector coupled to the HPLC system. Myricitrin was used as the standard for the flavonoid quantification in the argan leaves.

Statistical analysis

All experimental data reported as averages of 15 replicates. Data were analyzed using a one-way analysis of variance test facilitated by the STATISTICA 6 software package. Significant differences between means were determined using the Newman and Keuls test, at $p < 0.05$.

Results

Relative water content

The RWC analysis showed a difference between trees from the Bouizakarne and Ait Baha localities on the one hand, and those from Admine, Essaouira and Merght on the other (Table 2). The tree leaves from the Merght locality had the highest relative water content (77.82%) while those from Bouizakarne had the lowest content (55.09%).

Prolin content

The results showed significant differences (Table 2) for the prolin content among the seven localities. In fact, this amino-acid is a classical indicator of stress in plants (Rai, 2002). Indeed, prolin accumulation rate was higher in dryland plants, especially in leaves from Bouizakarne, with a content of 5.43 mg/g DW, followed by Ait Baha (4.56 mg/g DW), while leaves from Admine, which is subject to oceanic influences, had the lowest prolin content with only 1.3 mg/g DW.

Soluble sugar content

The analysis of the averages showed that the highest total sugar content was 9.64 mg/g DW in trees from Bouizakarne, followed by Merght with 7.89 mg/g DW. The lowest value was in Tamanar (6.76 mg/g DW), as shown in Table 2. There were significant differences between some of the studied localities.

Pigments content

The analysis of the leaf content of argan trees based on chlorophyll a and b and on carotenoids indicated a relatively quantity at Essaouira

for chlorophyll a (1.99 mg/g fresh weight; FW) compared to the other localities, while Bouizakarne had the lowest content (1.5 mg/g FW). Chlorophyll b was greatest at Ait Baha (0.71 mg/g FW) and lowest at Bouizakarne (0.53 mg/g FW). The highest carotenoids content was at Ait Baha (0.69 mg/g FW), while the lowest was at Admine (0.51 mg/g FW), as shown in Table 2.

There was no apparent significant difference between the studied localities for each of chlorophyll a, chlorophyll b and carotenoids.

The argan trees at the different localities appeared to respond in the same way to environmental conditions.

Protein content

The highest protein content was at Bouizakarne (505.21 mg/g DW), followed by Ait Baha (327.1 mg/g DW). The argan tree leaves from Tamanar and Imouzzar had the lowest protein content with an average of 211.1 mg/g DW. The analysis of variance for the total protein content indicated significant differences between leaves from some different localities (Table 2).

Total phenolic content

The results of the total phenolic contents analysis showed that there were significant differences between some of the studied localities (Table 3). Trees from Bouizakarne had the highest total phenolic content (8.76 mg/g DW), followed by Ait Baha (8.1 mg/g DW), while the lowest content was in leaves from Merght, Essaouira, Admine, Imouzzar and Tamanar.

Total flavonoids content

The quantification of total flavonoids based on the Andary method (1990) and Hariri et al. (1991) and a calibration range showed there were significant differences between some of the studied localities. The amounts of flavonoids obtained using the two assay methods was higher in the leaf extract from Bouizakarne (6.57 mg/g DW obtained by the Andary method), followed by Ait Baha (5.32 mg/g DW), while the lowest flavonoids content was registered in leaf extracts from Admine, Essaouira, Imouzzar, Tamanar and Merght (Table 3).

Table 2 Relative water, prolin, soluble sugar, protein contents and photosynthetic pigment content in argan leaves from the seven studied localities

Locality	RWC (%)	Prolin content (mg/gDW)	Soluble sugar content (mg/gDW)	Protein content (mg/gDW)	Pigment content (mg/gFW)		
					Chl a	Chl b	Carotenoids
Essaouira	76.53±6.36 ^c	2.06±1.73 ^a	7.24±2.56 ^{ab}	242.9±107.86 ^a	1.99±0.37 ^a	0.67±0.18 ^a	0.65±0.12 ^a
Tamanar	72.85±9.13 ^{bc}	1.69±1.14 ^a	6.76±1.99 ^a	211.1±108.11 ^a	1.75±0.45 ^a	0.66±0.17 ^a	0.63±0.14 ^a
Imouzzar	66.74±18.44 ^{bc}	2.2±1.14 ^a	7.73±2.19 ^{ab}	211.1±108.11 ^a	1.79±0.23 ^a	0.62±0.06 ^a	0.59±0.07 ^a
Admine	75.21±4.24 ^c	1.3±1.19 ^a	7.18±2.64 ^{ab}	317.1±136.9 ^a	1.55±0.45 ^a	0.56±0.18 ^a	0.51±0.12 ^a
Ait Baha	63.9±14.58 ^b	4.56±3.27 ^b	7.35±2.18 ^{ab}	327.1±252.8 ^a	1.92±0.31 ^a	0.71±0.13 ^a	0.69±0.11 ^a
Merght	77.82±6.46 ^c	1.9±1.23 ^a	7.89±2.74 ^{ab}	228.24±81 ^a	1.6±0.22 ^a	0.6±0.07 ^a	0.6±0.065 ^a
Bouizakarne	55.09±13.86 ^a	5.43±2.44 ^b	9.64±2.80 ^b	505.21±383.23 ^b	1.5±0.16 ^a	0.53±0.068 ^a	0.52±0.078 ^a

Mean values (± SD) in each column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Table 3 Total phenolic and total flavonoids contents and antioxidant activity in argan leaves from the seven studied localities

Locality	Total phenolic content (mg/g DW)	Total flavonoid content (mg/g DW)		Antioxidant activity (%)
		Calibration range	Andary method	
Essaouira	5.83±0.35 ^a	3,64±0.6 ^a	2,93±0,67 ^a	93.09±0.59 ^a
Tamanar	6.4±1.01 ^a	4,29±0.79 ^a	3,6±0,73 ^a	93.87±0.97 ^a
Imouzzzer	6.37±0.82 ^a	3,95±0.5 ^a	3,32±0,55 ^a	93.72±2.21 ^a
Admine	5.96±0.27 ^a	4,16±0.59 ^{ab}	4,33±0,82 ^a	94.29±0.43 ^a
Ait Baha	8.1±0.6 ^b	5,1±1.05 ^{bc}	5,32±1,2 ^{ab}	93.79±1.35 ^a
Merght	5.73±0.36 ^a	3,91±0.6 ^a	3,69±0,9 ^a	93.09±0.95 ^a
Bouizakarne	8.76±1.44 ^c	6,12±0.88 ^c	6,57±1,1 ^b	93.79±0.89 ^a

Mean values (± SD) in each column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Antioxidant activity

The results of DPPH test showed that extracts of argan leaves had very important antioxidant activity that was certainly related to the high amounts of phenolic compounds. There were no significant differences between leaf extracts among the studied localities (Table 3).

Chromatographic profiles analysis

The chromatographic profiles of leaf extracts from the various localities based on HPLC (Fig. 1) identified four major flavonoid molecules. The first molecule corresponded to myricetin 3-O-galactoside (retention time 3.2 min), the second molecule (retention time 3.9 min) to myricitrin, the third metabolite (retention time 5 min) was hyperoside and the last molecule (retention time 6 min) was quercitrin. Other minor compounds were detected using HPLC were myricetin and quercetin derivatives (Tahrouch et al., 2000).

The four major flavonoid molecules (myricetin 3-O-galactoside, myricitrin, hyperoside and quercitrin) were found consistently in argan leaf extracts from the seven studied localities.

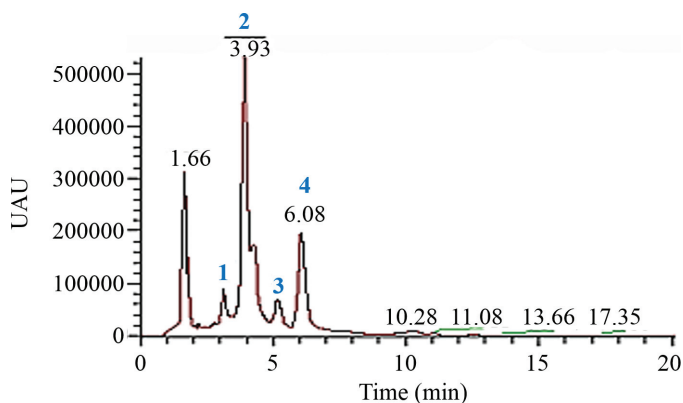


Fig. 1 High performance liquid chromatography results for methanolic extract from argan leaves

Discussion

Plant adaptation to environmental stress depends the characteristics of each species. Several studies have shown that plant resistance to drought is a result of morphological, physiological, biochemical and molecular mechanisms (Denden et al., 2008). From a physiological perspective, the current results showed that argan trees react to different levels of environmental stress.

The relative water contents in the trees from the seven studied localities indicated a water percentage decrease at Bouizakarne, Ait Baha and Imouzzzer, while the highest amounts were in leaves from Tamanar, Admine, Essaouira and Merght. The low content at the higher altitudes (Imouzzzer area) was in agreement with the results of Díaz-Barradas et al. (2010). The current results showed that the relative water content is low at the high altitudes and higher in the coastal areas (Tamanar, Admine and Essaouira). The leaf samples from Merght also had a high water content, despite its relative altitude; it was the ready availability of water there that resulted in the plants being able to retain such a high water content.

The comparatively low percentages at Ait Baha and Bouizakarne were probably due to their low rainfall, which led to tissue dehydration. Similar behavior was observed in several species subjected to water stress and especially Algerian argan trees (Berka and Aïd, 2009; Meslem et al., 2015) and wheat (Sassi et al., 2012).

Cellular dehydration can trigger other physiological mechanisms. Indeed, as soon as the argan trees start to feel environmental stress, osmotic adjustment mechanisms are activated by the accumulation of solutes (prolin and soluble sugars) to maintain cell turgor and physiological activities (Ge et al., 2014). A significant increase in the prolin content was recorded in trees at Ait Baha and Bouizakarne while the content of prolin decreased in the other localities. Bouizakarne also recorded the highest content of soluble sugars. These important contents of prolin and total sugars could be explained by high temperatures and low rainfall in these areas. Therefore, the increase in these solutes may have been due to the osmoprotective role against cellular dehydration when the plant's water potential is very negative (Yancey, 1994).

It appears that prolin can confer stress tolerance in plants by the development of an antioxidant system which can play an osmotic adjustment indicator role (Ranganayakulu et al., 2015). The prolin

could also have a role in cytoplasmic pH regulation (Denden et al., 2005) or constitute a reserve of nitrogen and carbon compounds, used later in plant growth during stress periods (Keller and Ludlow, 1993). It also plays a role in stabilizing membrane proteins (Errabii et al., 2006).

When the relative water content decreased, soluble sugars accumulated, as was the case at Bouizakarne. An increase in soluble sugars has been reported for many species evolving under different stresses such as argan trees (Berka and Aïd, 2009), potatoes (Farhad et al., 2011) and wheat (Johari-Pireivatlou, 2010). The accumulation of sugars is due to the degradation of starch reserves following their conversion into soluble sugars (Mohammadkhani and Heidari, 2008). Similar results were found in argan plants subjected to water deficit where the increase in the soluble sugar content was followed by a decrease in starch concentration (Berka and Aïd, 2009). Soluble sugars are osmolytes that can have a role in plant tolerance to environmental stress (Mohammadkhani and Heidari, 2008), such as at Bouizakarne which is the driest of the seven localities studied.

The accumulation of osmolytes in the leaves of argan trees seemed to maintain cell turgor; therefore the plant could keep its stomata open, to continue gas and water vapor exchanges as a part of photosynthesis and transpiration (Berka and Aïd, 2009).

On the other hand, the analysis of the two chlorophyll and the carotenoid rates showed no significant differences between the studied localities. During the current study, the chlorophyll and the carotenoid contents remained relatively unchanged regardless of the locality. Similar results have been reported in potatoes subjected to stress conditions (Farhad et al., 2011) and in carob (Fadel, 2011). According to Poljakoff-Mayber (1975), the ability to synthesize more chlorophyll under water stress is a criterion for the selection of drought-tolerant species.

The adaptation of the argan tree to stress involves phytochemical and physiological mechanisms. The argan tree reacts to environmental conditions by increasing the total proteins in the leaves; indeed, the total protein was high in the leaves from Bouizakarne while it was low in the other localities. Several authors have reported that plants subjected to environmental constraints synthesize specific proteins associated with stress, such as late embryogenesis abundant proteins (Arve et al., 2011; Meslem et al., 2015). These proteins provide complete protection of the cellular proteins (David and Grongnet, 2001) and the heat-shock proteins which permit the maintenance of protein and membrane structures of plant cells. The accumulation of leaf proteins under stress could also act as a nitrogen reserve used by plants after a dry period of summer (Bacelar et al., 2006).

The leaves from Bouizakarne and Ait Baha had the highest amounts of phenolic compounds (total phenols and total flavonoids), probably due to the arid climate in these two localities and their low rainfall compared to the others localities (Essaouira, Tamanar, Imouzzar, Admine and Merght). There were significant differences in the flavonoids content among the seven studied localities. Tahrouch (2000) reported that argan tree leaves from Imouzzar had the lowest flavonoid contents compared to those from Admine and Ait Baha. The increase in secondary metabolites content, in particular flavonoids,

could have been due to climate change (Jaakola and Hohtola, 2010). Therefore, the argan trees could have increased their phenolic components to adapt to changes in the environmental conditions (Tahrouch 2000; Fahmi et al., 2013). Plants synthesize phenolic compounds which can protect them from various adverse conditions (Ben Dkhil and Denden, 2010). Indeed, different studies have shown that the key enzymes in the polyphenol biosynthesis pathway were sensitive to these adverse conditions (Ma et al., 2014). The accumulation of these compounds could protect cells against free radicals and cell disruption during stress (Ben Dkhil and Denden, 2010).

The HPLC study showed the existence of four major flavonoids in the argan leaf extracts from the seven studied localities. Previous studies have also shown the existence of these same flavonoids in three localities of the Souss area (Imouzzar, Admine and Ait Baha) and in Oued Cherrat; these molecules (myricitrin, quercitrin, hyperoside and myricetin 3-O-galactoside) could constitute biochemical markers in argan trees (Tahrouch et al., 2000; Fahmi et al., 2013).

The antioxidant activity results of argan leaf extracts from the different localities had free radical scavenging percentages higher than 90%, as was also reported for argan trees by Joguet and Maugard (2013). The antioxidant activity of the argan leaves extract was certainly due to their important content of phenolic compounds. Generally, plants with great amounts of phenolics compounds have very strong antioxidant capacity. In fact, several authors have already demonstrated the close correlation between the phenolic compounds content and antioxidant activity (Popovici et al., 2009). Indeed, phenolics compounds are a major class of antioxidant agents because of their scavenging ability of free radicals (Shahidi et al., 1997). Due to their several hydroxyl groups, flavonoids have been shown to be highly effective scavengers of various free radicals implicated in several diseases (Das et al., 1990). The strong antioxidant activities could make plants suitable for a large spectrum of biological activities (Ćetković et al., 2007).

Conflict of Interest

The authors declare that there are no conflicts of interest

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