LC-MS PHENOLIC COMPOSITION CHANGES AND ANTIOXIDANT CAPACITIES OF THE SAHARAN TREE *ARGANIA SPINOSA* LEAVES UNDER SALNITY

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ABSTRACT

Adaptation of many plant species to hostile environmental conditions suggest the presence of powerful antioxidative constituents in their tissues such as phenolic compounds. Many works on antioxidant activity of the Moroccan argan oil have been carried out. However, it is the first time that salt impact on Algerian arganleaves is assessed. The main objective of this work was to study the soil salinity impact on phenolic content and composition, and the antioxidant activities of the argan leaves collected from three point in the same site of Tindouf region (Algeria) characterized by a gradient of salt concentration (Lightly Salt Tindouf, Salt Tindouf and Very Salt Tindouf). Variability of phenolic contents, antioxidant and free radical-scavenging activities of the argan leaves as function of salt soil concentration were evaluated. Identification was done by LC-MS system. Regarding phenolic contents (total polyphenol, flavonoid and condensed tannin), the Salt Tindouf leaves displayed the highest amounts (total polyphenol = 77.28 mg GAE/g DW). Moreover, the same tendency was observed for antioxidant activities, for instance, total antioxidant activity of leaves from Salt Tindouf was the highest (83.6 mg GAE/g DW). In addition, leaves from Salt Tindouf displayed the highest scavenging activity against DPPH radical (IC_{50} = 6.5 µg/ml) as compared to the two others points. These results were also confirmed by LC-MS analyses. Leaves synthesize more compounds with very important biological activities under salinity which allow them to be valorized in different fields, such as pharmacology and agro-food industries.

KEYWORDS: *Argan, phenol, salinity, antioxidant, LC-MS, Tindouf.*

1 INTRODUCTION

Arid and semi-arid lands represent one third of the earth's surface. In these areas, soil salinity is one of the limiting factors to plant productivity and crop yield. These ecosystems are characterized by irregular precipitations associated with significant evaporation increasing accumulation of salts in soil. Algeria is one of the affected countries, with almost 3.2 million hectares of saline surfaces. Therefore, a concerted effort to understand salinity effects on plants and to develop salt-tolerant species is essential to deal with soil salinization problems. Principally, salt stress causes an over reduction of photosynthetic electron chain and induce reactive oxygen species (ROS) occurrence (Hajlaoui et al. 2009) that lead to cellular damage and are known to be involved in several plant disruptions, as well as in senescence processes. ROS can also react with biological molecules, such as DNA, proteins, or lipids, generating mutations and damaging membranes, resulting into cell and tissue injuries. Biotic and abiotic stresses exert considerable influence on the secondary metabolite pool in plants. Enhanced synthesis of some secondary metabolites, as stressful condition responses, is believed to protect cellular structures from oxidation. In fact, adaptation of many plant species to hostile environmental conditions, suggest the presence of powerful antioxidative constituents in their tissues (Ksouri et al. 2007). Among the various kinds of natural antioxidants, polyphenolic compounds such as phenolic acids, flavonoids, and proanthocyanidins play a key role in plant adaptation to abiotic stresses and have received much attention. Particularly, when plants were submitted to saline treatment, variation in antioxidant pools, notably in polyphenols, was found. For instance, high salt treatment dramatically increased total polyphenol content in halophytic species such as *Cakile maritima* (Ksouri et al. 2007) as well as in glycophytes *Raphanus sativus* (Gaofenget al. 2010).

Argania spinosa from Sapotaceae family is an endemic species covering 96940 ha surface area in the extreme south-west of Algeria (region of Tindouf). This species belongs to the Saharan bioclimatic and naturally grows on poor soils characterized by a large range of salinity. The argan tree is known to be used as traditional remedy and for cosmetic purpose. In cosmetics, Argan oil is prized as moisturizing oil, against acne juvenile and skin flake off, as well as hair nourishment. This oil has also medicinal uses against rheumatism and burns healing. It is an aphrodisiac and has spermatogenetic property. The leaf infusion is used to treat gastritis, diarrhea, fever and headaches. In poultice, leaves treat sprains, infected wounds and scabby animals (El Babili et al. 2010). Actually, the oil derived from fruits could be of great interest to develop new prevention strategies against prostate cancer and cardiovascular disease (Adlouni 2009). Many works on antioxidant activity of the Moroccan argan oil have been carried out. However, it is the first time that salt impact on Algerian argan tree is assessed, especially on its antioxidant potentialities. This work aims to study salinity effect on antioxidant activities of argan tree leaves in relation with their phenolic profiles using LC-MS.

2 MATERIALS AND METHODS

2.1 Plant sampling

This work focuses on the Algerian argan tree (*Argania spinosa*) sampled from the region of Tindouf (south-west Algeria). The studied area belongs to the Saharan climate. In September 2012, a sampling campaign of argan leaves from three points in the same site with gradient soil salinity. Collected leaves were air dried then ground to obtain a fine powder.

2.2 LC-MS analyses

The extraction was performed with a soxhlet apparatus (type Gerhardt) as follow: Dried leaves (20 g) were packed into a filtration paper, inserted into a Soxhlet apparatus and extracted with 200 ml of pure methanol at 85°C. The extract gave a green colour which turned lighter through extraction until became colourless after 24h. All extract was retrieved and stored in the darkness at 4 °C until analysis. Sample was passed through a 0.45 µm nylon filter before injection into LC-MS. Separation of selected phenolic compounds was carried out using HPLC system (consisting of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 400 bar; Agilent 1100, Agilent technologies, Waldbronn, Germany) fitted with a reversed phase C18 analytical columnSuper-sphere 100. Column temperature was maintained at 30°C. The injected sample volume was 10 µL. Mobile phase A was milli-Q water consisted of 0.1% acetic acid and mobile phase D was acetonitrile. The optimised chromatographic condition was: 0–5 min, 2% D; 5–68 min, 100% D; 68–75 min, return to initial conditions. The flow-rate used was 0.250 ml/min. This HPLC system was connected to a mass spectrometer (Qtrap PerkinElmer), fitted with an electrospray interface operating in negative mode, using the following operating parameters: capillary voltage 3200 V; drying gas 423 l/Hr; gas temperature 300°C. LC/MS accurate mass spectra were recorded across the range 135–800 m/z. UV-Vis absorption spectra were recorded online during the HPLC analysis. The DAD detector was set to a scanning range of 190–800 nm. The phenolic compounds were identified mainly by their UV-spectra and MS spectra and by comparing with published data.

2.3 Salt soil analysis (electrical conductivity)

Soil electrical conductivity of the three points was determined using 50 g of the sifted soil of 2 mm with 250 ml of distilled water. The mixture was shacked for one hour and centrifuged during 10 min (2000 tours/min), then filtered and read using a conductivity meter.

2.4 Colorimetric quantification of phenolics

2.4.1 Determination of total polyphenol content

Colorimetric quantification of total polyphenol was determined as described by Dewanto et al. (2002). Hundred and twenty five micro liter of each sample extract suitably diluted was dissolved in 500 µl of distilled water and 125 µl of the Folin–Ciocalteu reagent. The mixture was shaken, before adding 1250 µl Na_2CO_3 (7%) and adjusting with distilled water to a final volume of 3 ml. After incubation for 90 min at 23°C in dark, the absorbance versus prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total phenolic content of leaves was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid (0–400 µg/ml). All samples were analyzed in triplicates.

2.4.2 Estimation of total flavonoid content

The total content of flavonoids was measured by a colorimetric assay according to Dewanto et al. (2002). An aliquot of the samples was added to test tubes containing 75 µL of a 5% $NaNO_2$ solution, and mixed for 6 min. Then, 0.15 ml of a freshly prepared 10% $AlCl_3$ solution was added. After 5 min at room temperature, 0.5 ml of 1N NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture without the sample as a blank. The concentrations of flavonoid compounds were calculated according to the equation that was obtained from the standard (+)-catechin graph, and were expressed as mg catechin equiv. g^{-1} DW (mg CE/g DW). All samples were analyzed in triplicates.

2.4.3 Total condensed tannins assay

The analysis of condensed tannins (proanthocyanidins) was carried out according to the method of Sun et al.(1998).To 50 µl of properly diluted sample, 3 ml of 4% methanol vanillin solution and 1.5 ml of concentrated hydrochloric

acid were added. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as mg CE/g DW. The calibration curve range was 0– 400 µg ml^{-1}. All samples were analyzed in triplicates.

2.5 Determination of antioxidant activities

2.5.1 Evaluation of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto et al. 1999). An aliquot of sample extract was combined in an eppendorf tube with 1 ml of reagent solution (0.6 Msulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank in UV-Visible spectrophotometer (Anthelie Advanced 2, SECOMAN). The antioxidant capacity was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW). All samples were analyzed in triplicates.

2.5.2 Scavenging ability on DPPH radical

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH quenching ability of *Argania spinosa* leaves extracts was measured according to Hatano et al. (1988). One milliliter of the extract at known concentrations was added to 0.5 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature in the dark for 30 min. The absorbance was then measured at 517 nm and corresponds to the extract ability to reduce the radical DPPH to the yellow-coloured diphenyl picryl hydrazine. BHT was a synthetic phenolic used as positive standard. The antiradical activity was expressed as IC_{50} (µg/ml), the antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0]^* 100 \quad (1)$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

2.5.3 Iron reducing power

Ferric reducing power is a simple and direct test of antioxidant capacity. This assay measures the electron-donating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants reduce the ferric ion/ferricyanide complex to the ferrous form. The capacity of plant extracts to reduce Fe^{3+} was assessed according to the method of Oyaizu (1986). Each extract was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 650g for 10 min. The upper layer fraction (2.5 ml) was mixed with deionised water and 0.5 ml of ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer and ascorbic acid was used as positive control. A higher absorbance might indicate a higher reducing power. EC_{50} value (mg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from linear regression analysis.

2.5.4 β-Carotene bleaching test (BCBT)

A slightly modified Koleva et al.(2002) method was employed to estimate Argania leave's capacity to inhibit the β-carotene bleaching. Two milligrams of β-carotene were dissolved in 20 ml chloroform and to 4 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated water was added, then the fresh emulsion was vigorously shaken. An aliquot (150 µL) of the β-carotene/linoleic acid emulsion was distributed in 96-well microtitre plates and methanolic solutions of the test samples or authentic standards (10 µL) were added. Three replicates were prepared for each concentration. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured using a model Biotek ELx808 at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in terms of blanching inhibition of the β-carotene using formula (2)

$$\beta\text{-Carotene bleaching inhibition (\%)} = [(S-C_{120})/(C_0-C_{120})] / 100 \quad (2)$$

Where C_0 and C_{120} are the absorbance values of the control at 0 and 120 min, respectively, and S is the sample absorbance at 120 min. The results were expressed as IC_{50} values (µg/ml).

2.6 Statistical analysis

Means were statistically compared using MINITAB 2000program with Student's t-test at $P < 0.05$ significance level. One-way analysis of variance (ANOVA) and Newman-Keuls multiple range test were carried out to test any significant difference between harvested points at $P < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Salt soil analysis

According to salinity soil scale mentioned by Aubert (1978), results showed that our site was divided into three classes based on soil salinity. The first point called lightly salt Tindouf (L-ST) was characterised by a slightly salted soil (Table 1). The second area was salty (Salty Tindouf, ST) and the third one in which the soil was very salty is

called Very Salty Tindouf (V-ST).

3.2 Total polyphenol, flavonoid, and condensed tannin contents

In this study, total phenol, flavonoid, and condensed tannin contents were estimated by colorimetric methods based on the absorbance values. Leaves from the three points were harvested from the same site (Tindouf region) and at the same time to avoid climatic influence on the antioxidant potential.The main factor that differs between the three points is the soil electrical conductivity due to salt concentration. Results established that studied arganian leaves showed a significant intra-specific variability in their total polyphenolic, flavonoid and condensed tannin contentsas function of salt soil according to one way (ANOVA) statistical analysis (Table 2).

Regarding the amount of total phenolic, (ST) leaves showed the highest total phenol content, reaching 77.28 mg GAE/g DW, followed by lightly salted Tindouf of (L-ST) (50 mg GAE/g DW) and at last (V-ST) with 29.91 mg GAE/g DW. In comparison with others medicinal species, total phenolic content of A. spinosa was significantly higher as compared to the halophyte Tamarix gallica (34.44 mg GAE/g DW) and to the glycophytic Nigella sativa L. (10.04 mg GAE/g DW) (Bourgou et al. 2008; Ksouri et al. 2009). Increasing in the total polyphenol content between (L-ST) and (ST), is more likely related to the difference in soil salinity. Previous studies have shown that plant phenolic contents and their antioxidant activities depend on biological factors (genotype, organ and ontogeny), as well as environmental (temperature, salinity, water stress and light intensity…) conditions (Ksouri et al. 2008). In this case, phenolic compounds play a key role in plant adaptation to abiotic stresses. Their gradients in concentrations within-species may reflect different requirements for dealing with abiotic stresses (Hajlaoui et al. 2009). Consequently, the biosynthesis of these metabolites can be significantly modulated by salt stress depending on plant salt sensitivity (Kim et al. 2008). With this respect, our results are similar to those found by Jeong-Ho Lim et al. (2012) which mentioned that total phenolic content in Fagopyrum esculentum sprouts, treated with 10, 50, and 100 mM NaCl, were respectively 57, 121, and 153% higher than control sprouts (128 mg GAE/g DW). Moreover, the same tendency was also observed by Gaofeng et al. (2010) on radish sprouts treated with 0 and 100 mM, with a significant augmentation of total phenolic amounts (75 and 92 mg GAE/g FW, respectively). Additionally, Ksouri et al. (2007) reported a significant increase in the halophyte Cakile maritima polyphenols under salt treatment. In fact, at control plants (0 mM NaCl), total phenolic content was estimated at 43 mg GAE/g DW and this amount reach to 67 mg GAE/g DW at the higher salt concentration (100 mM NaCl). However, the decrease of polyphenols in (V-ST) leaves suggests the limit of tolerance of argan tree and salt sensitivity behaviour at a very higher concentration of soil salt. This data is confirmed by Bani-Aameur&Sipple-Michmerhuizen (2001) which mentioned that *Argania spinosa* tolerate salt but at moderate concentration in the soil. This is typical tolerant glycophyte behaviour, requiring the presence of moderate salt concentration to express maximal growth potentials. In fact, they state that 7.5g/l NaCl would be the upper limit of salt concentration that argan seedlings could tolerate. The same variability was found for flavonoid content. In fact, leaves from (ST) plants were the most provided on these compounds as compared to the two others (Table 2). Leaf flavonoid contents were equal to 21, 13 and 6 mg CE/g DW, respectively for (ST), (L-ST) and (V-ST) plants. A comparable result was mentioned by Hajlaoui et al. (2009) in Zea mays which found that flavonoid content increased with salt stress. Moreover, results showed that Argania spinosa leaves displayed a higher content of these pigment compounds as compared to others medicinal plants such as *Mesembryanthemum crystallinum* (0.31 mg CE/g DW) and *Mesembryanthemum nodiflorum* (0.45 mg CE/g DW) (Falleh et al. 2009). Alike tendency was also recorded for tannin content in argan leaves. The richness of (ST) tree (5.31 mg CE/g DW) is marked, and respectively about 4- to 11-folds higher in comparison to (L-ST) and (V-ST) trees (Table 2). Elfeel et al. (2012) mentioned that condensed tannin contents were significantly variable depending on salt concentration applied to *Acacia saligna* (0.9 and 1.1 % for control and treated plants, respectively). Elfeel et al. (2013) showed the influence of salt stress on condensed tannin accumulation in *Balanites aegyptiaca*. They found that for the control plants, the amount of tannins was estimated to 0.43g/100g while in treated plants (CE=8 dS/m), this value increases to 0.52g/100g. In addition, Falleh et al. (2013) displayed that climatic constraints led to the biosynthesis of molecules with higher degree of polymerization (DPn) mainly condensed tannins.

3.3 Antioxidant activities of leaf extracts

3.3.1 Total antioxidant activity

The antioxidant protective effect of *Argania spinosa* leaves was measured by several, in vitro, methods. For total antioxidant activity, the phosphomolybdenum assay is a quantitative method to evaluate total antioxidant capacity. Results revealed significant differences in total antioxidant activities of the three points of *A. Spinosa* leaves related to their salinity levels (Table 3).

Leaf total antioxidant activity was the highest in ST (83.6 mg GAE/g DW), followed by L-ST (58.4 mg GAE/g DW) and was reduced by the half in V-ST (42.0 mg GAE/g DW). Moreover, in comparison with earlier data, total antioxidant activity of *A. spinosa* leaves extracts was 6 times higher than in the halophyte *Tamarix gallica* leaves, which was equalto14.66 mg GAE/g DW (Ksouri et al. 2009). This strong antioxidant activity of *A. Spinosa* leaves might be attributed to the presence of powerful antioxidants such as phenolic compounds. In fact, some studies have shown, for several fruits and vegetables as well as for medicinal plants (Bourgou et al. 2008), that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity. In fact, the difference in

total antioxidant activity between the three points is more likely linked to the difference in their leaf phenolic biosynthesis enhanced by salt stress (Jeong-Ho Lim et al. 2012).

3.3.2 DPPH radical scavenging test

Similarly, the scavenging effect of (ST) leaf extract, against the synthetic radical DPPH, was significantly more potent as compared to those of (L-ST) and (V-ST) as shown in Table 3. Actually, IC_{50} value of (ST) was about 2- and 4-folds lower than (L-ST) and (V-ST), respectively. Moreover, (ST) leaves exhibited the best IC50value (6.5 µg/ml) which was statistically even more powerful than the positive control (BHT, IC_{50} = 11.5 µg/ml). The comparison with previous work focusing on the same plant showed that argan leaf extracts are more efficient than fruits ones with an IC_{50}value equal to 32.3 µg/ml (El Babili et al. 2010). In addition, this antiradical activity of *A. spinosa* extracts was better than other medicinal halophytes, such as *Salicornia herbacea* (IC_{50} = 55.3 µg/ml) (Essaidi et al. 2013) and medicinal glycophytes like Pisonia alba and *Centella asiatica* (IC_{50} = 175 µg/ml and 200 µg/ml, respectively) (Subhasree et al. 2009). Moreover, it is worth to notice that antioxidant activity of A. Spinosa was increased with salinity that induces the biosynthesis of phenolics. Therefore, these results confirmed the study of Jeong-Ho Lim et al. (2012) which suggested that phenolic compounds produced under salt stress are more likely powerful antioxidants. Ksouri et al. (2007) comparatively analysed leaf polyphenol content and antioxidant activity in two C. maritima Tunisian accessions (Jerba and Tabarka, respectively) under salt constraint. Since the tolerance of Jerba plants to mild NaCl treatment was concomitant with shoot enrichment in polyphenols, while the relative sensitivity of Tabarka provenance to salt was paralleled at shoot phenolic decrease. Therefore, authors hypothesize that the difference in capacity to accumulate polyphenols participated in the difference in salt tolerance of the two varieties. In addition, Navarro et al. (2006) showed increased total phenolic content with moderately saline level in red peppers. In fact, phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals.

Therefore, the decreased antiradical activity in (V-ST) leaves (IC_{50}= 26.5 µg/ml) is probably linked to phenolic compounds decrease which suggests the salt sensitivity of *A. spinosa* at high NaCl medium (Bani-Aameur&Sipple-Michmerhuizen 2001).

3.3.3 Ferric reducing antioxidant power (FRAP)

In the reducing power assay, the presence of reductants (antioxidants) in the extract would result in Fe^{+3}/ferric cyanide complex reduction to ferrous form by donating an electron (Sahreen et al. 2010). Considering the Fe-reducing power of *A. spinosa*, as for previous antioxidant tests, (ST) leaves showed the highest capacity to reduce iron (EC_{50}= 239 µg/ml) as compared to (L-ST) and (V-ST) which both showed an EC_{50} values over to 330 µg/ml (Table 3). Moreover, *A. spinosa* extracts depicted an interesting reducing power compared to other plants such as Salsola kali (EC_{50} = 457.66 µg/ml) and *Suaeda maritima* (EC_{50} = 660 µg/ml) (Ksouri et al. 2008; Oueslati et al. 2012). Although, the positive control (ascorbic acid) showed stronger activity (EC_{50} = 40µg/ml) in comparison to argan leaf extracts. The potent Fe^{2+}-reducing capacity exhibited by (ST) leaves may be related to the presence of powerful antioxidants in this extract such as flavonoids that can release electron. In this context, several studies have correlated antioxidant activity with total phenolic or total flavonoid amounts (Li et al. 2009).

3.3.4 β-carotene linoleic acid bleaching assay

β-carotene undergoes rapid discoloration in the absence of antioxidant compounds, therefore, the presence of antioxidants could inhibit the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jianxiong et al. 2008). The ability of argan leaves (L-ST, ST and V-ST) to inhibit β-carotene bleaching, was presented in Table 3. According to data presented in this table, this imperative activity was also differs greatly depending on soil salinity. Yet, in contrast with previous antioxidant tests, (V-ST) leaf extracts exhibit the powerful activity and the argan leaf efficiency were ranged as followed: V-ST (IC_{50}= 863 µg/ml), ST (IC_{50}= 1663 µg/ml) and L-ST (IC_{50}= 2000 µg/ml). This result can be explained by the presence of specific antioxidant phenolics in (V-ST) leaves such as rutin (Jianxiong et al. 2008), which plays an important role in inhibiting the bleaching of β-caroteneand to preserve membrane integrity of leaf and their components against lipid peroxidation.

Moreover, used as positive controls, BHA and BHT appeared to be the most powerful inhibitors, with the lowest IC_{50} values (48 and 75 µg/ml, respectively).

3.4 LC-MS identification of bioactive secondary compounds

The analysis of chromatograms, in negative mode, of A. spinosa leaf extracts, from three different salt points, by LC-MS revealed the presence of 15, 7 and 6 phenolics in (ST), (L-ST) and (V-ST) leaves, respectively. Among them, 8flavonoid compounds were characterized and further identified by referring to the literature which reported their occurrence in this species. Total ions chromatograms (TIC) obtained are illustrated in figure 1.

The identified flavonoids in the argan tree leaves are distributed as follows: 6 compounds in (ST) leaves, and respectively 4 compounds in (L-ST) and (V-ST) leaves. For example, epicatechin (only in ST) ($C_{15}H_{14}O_6$,m/z=289.29) (Figure 1.c, Peak2) (Charrouf et al. 2007); Myricetin-3-O-galactoside (only in L-ST and ST) ($C_{21}H_{20}O_{13}$, m/z = 479.43) (Figure 1.b,c, Peak 4) (Joguet&Maugard 2013); Rutin (only in V-ST) ($C_{27}H_{30}O_{16}$, m/z= 609.71) (Figure 1.a, Peak6), the identification of this molecule was confirmed using standard; Hyperoside/isoquercitrin (in the three

points) ($C_{21}H_{20}O_{12}$, m/z= 463.41) (Figure 1.a,b,c, Peak7) (Charrouf et al. 2007); Quercetin-O-pentose/Naringenin-7-O-glucoside (in the three points) ($C_{20}H_{18}O_{11}/C_{21}H_{22}O_{10}$, m/z= 433.40) (Figure 1.a,b,c, Peak8),(Charrouf et al. 2007); Quercitrin (in the three points) ($C_{21}H_{20}O_{11}$, m/z= 447.39) (Figure 1.a,b,c, Peak9), (Joguet&Maugard 2013); Naringenin (only in ST) ($C_{15}H_{12}O_5$, m/z= 271.13) (Figure 1.c, Peak10) the identification of this molecule was confirmed using standard. Some molecules were not identified so it can correspond to new compounds. All these phenolic compounds mentioned are summarized in Table 4, with their molecular formula ($C_xH_yO_z$), selected ion ([M-H]$^-$m/z), UV absorbance (λ_{max}).

In some cases, argan leaves from the three points contain the same molecules, some molecules are present in two points and absent in the third one and others have been reported only in one point. The three points contain: Hyperoside or isoquercitrin, Quercetin-O-pentose or Naringenin-7-O-glucoside and Quercitrin. This last molecule is one of the most abundantly and widely distributed antioxidant molecules in the edible and medicinal plants which exhibit a wide range of biological activities, such as antioxidant (Materska&Perucka 2005). Some studies have shown that Hyperoside possess cytoprotective effects against oxidative stress (Hosseinimehr et al. 2008) and a very important reducing power (Rainha et al. 2013). Isoquercitrin is considered as a powerful antioxidant and exhibited strong DPPH radical activities (Xingping et al. 2013). The fact that the argan leaves have an important antioxidant activity in the three points compared with other plant species is probably related to the presence of these biomolecules. β-carotene bleaching inhibition in (V-ST) is higher in comparison with the two others, and conversely for other activities, is probably related to the presence of rutin, as some studies have shown that the presence of rutin could inhibit the extent of β-carotene bleaching by neutralizing the linoleate-free radical (Jianxiong et al. 2008). (ST) differs from the two others points because, in addition to the molecules mentioned above, it contains two other molecules that are absent in (L-ST) and (V-ST), which are Epicatechin and Naringenin. Epicatechin has been suggested to be one of the most effective antioxidants among several secondary metabolites (Ishige et al. 2001), better radical scavengers and reducing compounds than usually recognised antioxidants like α-tocopherol (Montserrat Duenas et al. 2010). Naringenin is considered to have various bioactivities effect as antioxidant and reactive oxygen species (ROS) scavenger (Turkkan et al. 2012). Thus, the important antioxidant capacity manifested through (ST) leaves is related to the presence of these two powerful antioxidant compounds added to other phenolic molecules.

4 CONCLUSION

This work could be considered as the first study on antioxidant activities and phenolic identification of the Algerian argan leaves. Main results reveal a significant variability between the three points related to leaf phenolic composition as response to soil salt concentration. Moreover, phenolic compounds identified in *Agania spinosa l*eaves exhibit potent antioxidant activities (DPPH free radical scavenging, β-carotene bleaching inhibition) and also have a good iron-reducing power. These features are further increased depending on medium salinity. Moreover, the edible leaves of argan, mainly from (ST) points, are a good source of potent flavonoids with very important biological activities which allow them to be valorized in different fields such as: pharmacologic, medicinal or agro-alimentary.

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Table 01: Electrical conductivity (EC) of the three points harvested from Tindouf region (L-ST, ST, V-ST)

Salinity class	Unsalted	Lightly salted	Moderatly salted	salted	Very salty
EC (mmhos/cm)	<0.6	0.6 to 1.2	1.2 to 2.4	2.4 to 6	> 6
Total salt (g/l)	<0.36	0.36 to 0.72	0.72 to 1.44	1.44 to 3.6	>3.6
Lightly Salt Tindouf (L-ST)		0.8			
Salt Tindouf (ST)				4.35	
Very Salt Tindouf (V-ST)					8.5

Table 02: Total polyphenol (expressed as mg of GAE/g DW), flavonoid and condensed tannin contents (expressed as mg CE/g DW) of Argania spinosa leaves. Values (means of triplicate repetition) followed by at least one same letter within a raw were not significantly different at p< 0.05

Argania spinosa point harvested	Polyphenols	Flavonoids	Condensed tannins
L-ST	48.07 **b**	12.63 **b**	1.33 **b**
ST	77.28 **a**	20.84 **a**	5.31 **a**
V-ST	29.91 **c**	5.97 **c**	0.46 **c**

Table 03: Total antioxidant capacity (mg GAE/g DW) and antioxidant capacities against, DPPH., β-carotene bleaching activities (IC50 in µg/ml) and iron reducing power (EC_{50} in µg/ml) of Argania spinosa leaf extracts and authentic standards (BHT, BHA and ascorbic acid) expressed in IC_{50} value (µg/ml). Values (means of triplicate repetition) followed by at least one same letter within a raw were not significantly different at p< 0.05

	Total antioxidant capacity	DPPH test	Iron reducing power	*β*-Carotene bleaching activity
L-ST	58.38**a**	15.5**b**	332**b**	2000**a**
ST	83.64**b**	6.5**d**	239**c**	1663.3**b**
V-ST	42.04**c**	26.5**a**	450**a**	863.3**c**
BHT	-	11.5**c**	-	75**d**
BHA				48**d**
Ascorbic acid	-	-	40**d**	-

Table 04: Tentative identification of phenolic compounds in Argania spinosa leaves from different points (L-ST, ST, and V-ST) by LC-ESI-MS

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	Molecular Formula	Tentative Identification	Point sampling
1	15.437	206,275	305.30	-	NI	ST
2	18.101	204,275	289.29	$C_{15}H_{14}O_6$	Epicatechin	ST
3	18.758	224,280	497.46	-	NI	V-ST
	18.657					L-ST
	18.619					ST
4	20.081	206,258,356,262	479.43	$C_{21}H_{20}O_{13}$	Myricetin-3-O-galactoside	L-ST
	20.072					ST
5	20.488	206,258,354	595.58	-	NI	L-ST
6	20.90	206,258,356	609.71	$C_{27}H_{30}O_{16}$	Rutin	V-ST
7	21.283	210,262,350	463.41	$C_{21}H_{20}O_{12}$	Hyperoside/isoquercitrin	V-ST
	21.339					L-ST
	21.219					ST
8	22.467	206,256,354	433.40	$C_{20}H_{18}O_{11}$ $C_{21}H_{22}O_{10}$	Quercetin-O-pentose Naringenin-7-O-glucoside	V-ST
	22.467					L-ST
	22.403					ST
9	22.958	206,256,348	447.39	$C_{21}H_{20}O_{11}$	Quercitrin	V-ST
	23.106					L-ST
	23.022					ST
10	28.19	196,212,290	271.13	$C_{15}H_{12}O_5$	Naringenin	ST
11	34.474	-	325.34/555.60	-	NI	V-ST
	34.418					ST
12	38.858	-	473.62	-	NI	ST
13	40.190	192,236,275	277.44	-	NI	ST
14	52.854	-	793.89	-	NI	ST
15	56.64	-	255.50	-	NI	V-ST
	56.637					ST

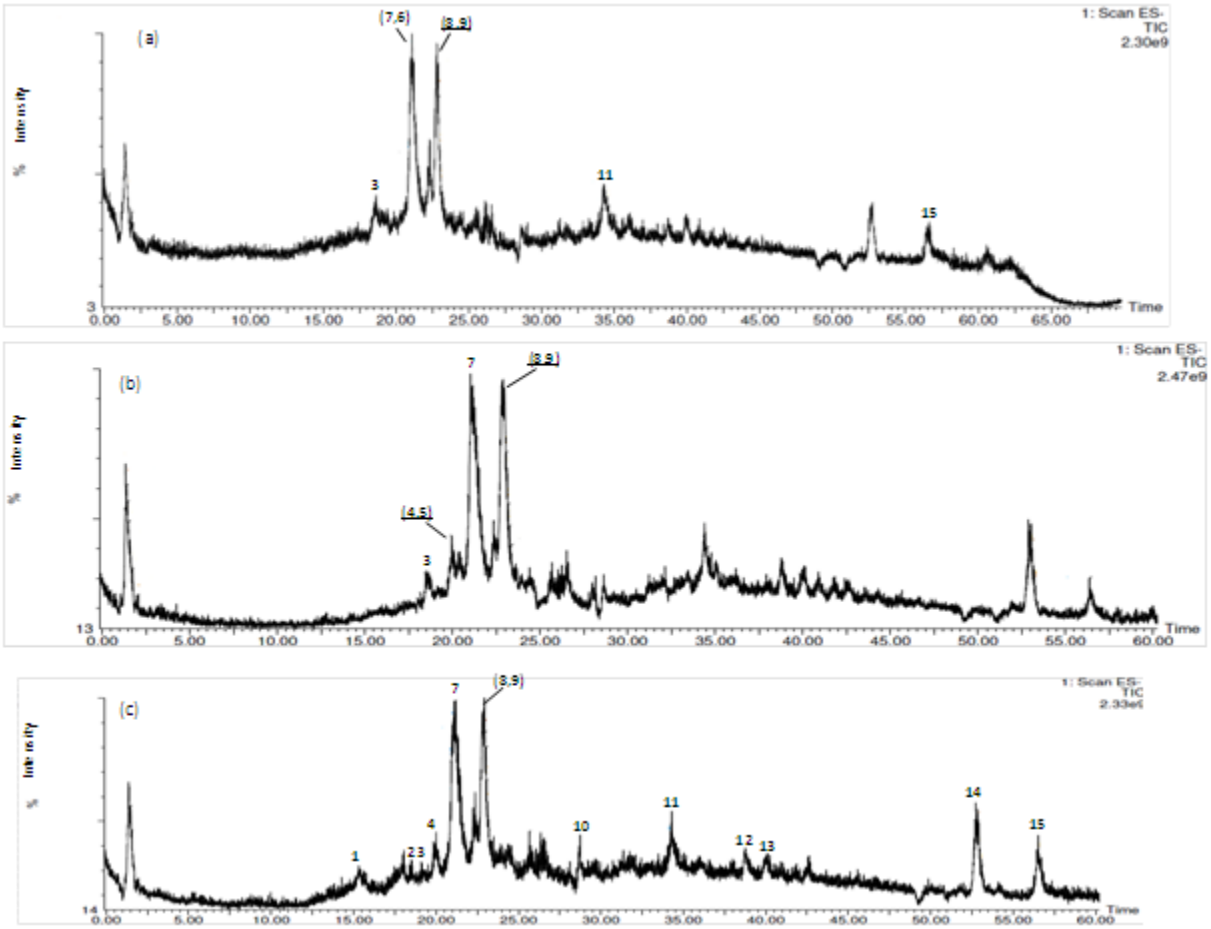

Figure 01: Total Ion Chromatograms (TIC) of leaf extracts of Argania spinosa from three points related to soil salinity : (a) A. spinosa from Very Salt Tindouf (V-ST), (b) A. spinosa from Lighltly Salt Tindouf (L-ST), (c) A. spinosa from Salt Tindouf (ST). Peak identities are numbered in Table 4