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Nitraria Retusa: Potential Source of Bioactive Molecules and Antioxidant Capacities

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Abstract: *Nitraria retusa plant samples were collected at the sebkha of El Kélbia, Kairouan and phenolic extracts with 70% ethanol were prepared. Quantification of total polyphenols, flavonoids and tannins was performed. The results showed the presence of 48.61 mg EAG / g MS for the first group and, respectively 50.53 and 2.7 mg EC / g MS for the flavonoids and the condensed tannins. On the other hand, the shoot extract of N. retusa has an important antioxidant power, first against the DPPH radical (63 µg / ml), then as an iron reducing power (0.92 mg / ml), then as an inhibitor of bleaching of β-carotene (59% for an extract used at 1 mg / ml) and finally capable of reducing molybdenum (VI) ions to molybdenum (V) (166.11 mg EAG / g MS). On the other hand, the content of MDA relative to leaf extracts is equal to 25.55 nmol / g MF and the percentage of electrolyte leakage is 65.4%. Other plant substances were measured with contents equal to 6.16 and 3.08 mg / ml for chlorophylls a and b, 2.05 mg / ml for carotenoids, 0.145 µg / ml for anthocyanins and 5, 68 mg / g MF for proline. Considering the antioxidant potential of the extract of N. retusa, it was proposed to determine the phytochemical profile of its leaf extracts by HPLC. Several phenolic compounds are determined such as catechol, catechin, hydroxycinnamic acid, hyperoside, kaempferol-3-O-rutinoside, trans-cinnamic acid and isorhamnetin-3-O-glucoside.*

Keywords: *Antioxidants ; Nitraria retusa ; Phenolic compounds ; Phytochemistry*

1. INTRODUCTION

The various constraints linked to the climate (high heat or cold), to the soil (salinity, drought and contamination in heavy metals) and to pests (attacks by insects) imposed on the plant are at the origin of different physiological manifestations such as high production of free radicals like reactive oxygen species (ROS) which cause a redox imbalance known as oxidative stress **[1].** In the leaves, this production of radicals like superoxide (O2−) is produced thanks to a transfer of electrons at the level of the respiratory systems PSI and PSII of the chloroplast by reduction of oxygen O2 and by bringing in chlorophyll **[2]**. Moreover, pigments such as chlorophylls (in chloroplasts) and proline as well as carotenoids (light collectors, liposoluble and located in chromoplasts) and anthocyanins (water-soluble pigments in vacuoles) are considered as indicators of stress. Among the damage observed in the plant cell, we can cite the disruption of membrane permeability characterized by the peroxidation of membrane lipids, essentially unsaturated fatty acids, with the production of different aldehydes such as malondialdehyde (MDA) which leads to DNA damage, causing dysfunction or even cell death **[1]**. To eliminate these ROS, halophytes, as examples of plants that tolerate salinity, are equipped with systems including the production of osmolytes (such as proline) and also polar antioxidant molecules such as polyphenols **[3,4,5].** Among the phenolic compounds, mention may be made of phenolic acids, flavonoids and tannins (hydrolyzable and condensed). All phenolic compounds have different structures characterized by the presence of a benzoic ring which gives them different mechanisms of antioxidant properties that prevent the propagation of redox reactions by neutralizing free radicals **[6]).** Several works have worked on the extraction of these phenolic compounds and their antioxidant activities such as those which act directly or indirectly on the radical **[3,4,6,7,8**). Aside from these antioxidant effects, extracts rich in phenolic compounds have shown multiple biological activities such as antimicrobial effects **[9]** as well as anticancer effects **[7,8]** and anti-inflammatory **[10,8]**

In this present work we propose to study the antioxidant capacity of *Nitraria retusa*, a North African halophyte of the Nitrariaceae family (formerly belonging to the Zygophyllaceae) well adapted to the arid climate thanks in particular to its fleshy leaves and also known in Tunisia under the name of 'Ghardak'. A previous study was carried out of this plant by some authors of this present work in order to enhance the role in the control of cellular accumulation of fat **[11]** A second study also revealed the cardioprotective role associated with antioxidant effects relating to the extract rich in anthocyanins from the juice of *Nitraria tangutorum* **[12]** In addition, the fruits of *N. retusa* are also known as a treatment for hypertension and difficult menstruation **[13]** Regarding the phytochemical profile, the genus *Nitraria* has long been known for its richness in polyphenols, mainly the group of flavonoids, with isorhamnetin as key molecule with strong biological activities, particularly antioxidant and antiproliferative **[13]**

Thus, the aim of this work is to further explain the origins of the traditional benefits of *Nitraria retusa* by analyzing its antioxidant profile by measuring the leaf contents of total polyphenols, flavonoids and condensed tannins with a focus on the phytochemical aspect. Measurements of some antioxidant activities such as anti-free radical capacity against DPPH, total antioxidant capacity, reducing power and the inhibitory effect of *β*-carotene bleaching are present in this work.

2. MATERIALS AND METHODS

2.1. Sampling and Extraction

The leaves of the halophyte *Nitraria retusa* were collected in August 2016 at the sebkha of El Kélbia, Kairouan. This extraction was carried out by mixing 10 grammes of the plant powder with 100 ml of 70% ethanol solvent. The mixture is then stirred for 30 min and kept in the dark at 4 ° C for 24 hours. After centrifugation for 15 min at 1000xg, the extract was filtered using a 0.22 µm Millipore filter. The solvent was evaporated using a rotary evaporator and then the extract was stored at 4 ° C for further experiments. **[4]**

2.2. Determination of Secondary Metabolites

The determination of total polyphenols is carried out with the Folin-Ciocalteu reagent **[4]** which, in an alkaline medium, is reduced to tungsten and molybdenum oxide giving a blue color in the presence of polyphenols. An aliquote of 0.125 ml of the suitably diluted extract is added to 0.5 ml of distilled water and 0.125 ml of the Folin-Ciocalteu reagent. Optical density is measured using a spectrophotometer at a wavelength of 760 nm. Three replicates were performed. The standard curve is prepared with gallic acid at concentrations of 50, 100, 200, 300, 400 and 500 mg / l. The contents are expressed in mg of gallic acid equivalent per gram of dry matter (mg EAG / g DM).

The determination of the total flavonoids was carried out according to the method of **Zar Kalai et al. (2013) [4]** and which is based on the formation of the complex between flavonoids and aluminum chloride (0.150 ml of AlCl3,6H2O, 10%). This presence is demonstrated with NaOH (0.5 ml to 1M). The absorbance value was taken at a wavelength of 510 nm. The intensity of the orange color indicates the importance of the flavonoid content of plants. The standard curve is prepared with catechin at concentrations ranging from 50 to 400 mg / ml. The flavonoid contents are expressed in mg of catechin equivalent per gram of dry matter (mg EC / g DM).

In the presence of 1.5 ml of concentrated hydrochloric acid (HCl), the condensed tannins are depolymerized and by reaction with 3 ml of vanillin (diluted in 4% methanol) are transformed into anthocyanidols of red color, measurable by spectrophotometry at 500 nm **[4]** As with flavonoids, the standard is catechin. The tannin contents are expressed in mg of catechin equivalent per gram of dry matter (mg EC / g DM).

The MDA assay is performed according to the protocol using thiobarbituric acid **[5]** Malondialdehyde or MDA is an oxidized lipid resulting from the peroxidation of lipids, polyunsaturated fatty acids, of the membrane. After grinding 250 mg of fresh *N. retusa* leaf in the presence of liquid nitrogen or acid (TCA) and centrifugation, a test portion of 250 µl is added to 1 ml of a solution composed of

thiobarbituric acid (TBA, 0.5%) and trichloroacetic acid (20%). After incubation at 95 ° C for 30 min during which the aldehydic compounds, essentially malondialdehyde, will react with TBA to form the TBA-MDA complex, the reaction is adjusted to 2 ml and stopped by cooling. The absorbance of the mixture obtained after centrifugation at 10,000 g for 15 min was measured at 532 nm (non-specific absorbance) and 600 nm. The optical density is then corrected by subtracting OD532 from OD600. The concentration of MDA in the fresh material, expressed in nmol / g MF, is then calculated using its extinction coefficient (ε = 155 mM-1cm-1).

The conductivity of the plant solution is estimated by the percentage of electrolyte leakage. For this, fresh leaves of *N. retusa* (200 mg) are excised into small discs and placed in 10 ml of bi-distilled water for 3 hours, at 37 ° C. After incubation, the conductivity is measured with a conductivity meter. The solution is then incubated at 95 ° C for 30 min, then the conductivity is measured again.

The extraction and determination of proline, which is an amino acid necessary for osmotic adjustment, is carried out according to the method of Bates et al. (1973). First, the leaves (20 mg) are ground in an acidic solution such as 3% (w / v) aqueous sulfosalicilic acid. Then, the mixture is centrifuged for 15 min at 14,000 x g and the supernatant is mixed with 1 ml of 1% (w / v) ninhydrin in 60% (v / v) acetic acid. After 1 h in a boiling water bath, the reaction is stopped by cooling in ice. Finally, 2 ml of toluene are added and the whole is vortexed for 2 min before incubation at room temperature. The assay is subsequently carried out at 520 nm. The proline contents are determined by referring to a standard curve (5 to 30 µg / ml).

For the determination of the photosynthetic chlorophyll pigments (a and b) and carotenoids, fresh leaf material of the order of 120 mg was placed in a vial containing 5 ml of 80% acetone **[5]** After a one week incubation in a cold room at 4 ° C and in the dark followed by filtration, optical density readings at the following wavelengths : 470 nm, 645 nm and 662 nm were taken at using a type 8 auto Cell UVS-2700 dual-beam visible UV spectrophotometer.

The anthocyanin content of aerial parts was determined according to the method of **Ehlenfeldt and Prior (2001)**. **[14]** Indeed, the fresh leaves of the order of 120 mg are incubated in the presence of a mixture of solvents : HCl-H2O-MeOH (1//3/16) in the dark and at 4 ° C for 72 hours. Absorbance was measured at 530 and 653 nm. The anthocyanins soluble in this solution exhibit a maximum absorption spectrum at 530 nm, the subtraction of 0.24 × A653 compensates for the excess absorption at 530 nm due to the presence of the chlorophylls.

2.3. Edaphic Analysis

The collected soil is dried at 60 ° C until a stable dry matter mass is obtained. The extraction was carried out by mixing 10 grammes of soil with 25 ml of distilled water. After 30 min of stirring, the solution was centrifuged at 3000 rpm for 20 min then filtered and adjusted to 25 ml. The determination of the pH of the soil solution, as well as the electrical conductivity (expressed in mS / cm) were carried out.

2.4. High Performance Liquid Chromatography (HPLC) Phytochemical Analysis

Prior to injection into the HPLC system, the extract was passed through a 0.45 µm nylon filter to remove impurities. The determination of the phenolic compounds was carried out using an HPLC system (consisting of a vacuum degasser, an autosampler and a binary pump with a maximum pressure of 600 bar ; Agilent 1260, Agilent technology, Germany) equipped with a C18 reverse phase analytical column with a particle size of 4.6x100 mm and 3.5 µm (Zorbax Eclipse XDB C18). The temperature of the column was maintained at 25 ° C. The volume of sample injected was 2 µl and the mobile phase flow rate was 0.4 ml / min. Mobile phase B was milli-Q water consisting of 0.1% formic acid and mobile phase A was acetonitrile. The proportions between the two phases over time were as follows : 10% A, 90% B (0 min) ; 20% A, 80% B (5 min) ; 30% A, 70% B (10 min) ; 50% A, 50% B (15 min) ; 70% A, 30% B (20 min) ; 90% A, 10% B (25 min) ; 50% A, 50% B (30 min) ; 10% A, 90% B (35 min). UV absorption spectra were recorded on a computer during HPLC analysis. A high sensitivity detector (DAD-HS) was set to a scanning range of 200 to 400 nm. The identification of the peaks was obtained

by comparing the retention time and the UV spectra of the phenolic chromatogram of *N. retusa* with those of the available standards. The desired compounds are detected at a wavelength of 280 nm. **[8]**

2.5. Evaluation of Antioxidant Activity

2.5.1. Anti-Radical Power (DPPH)

The DPPH radical (2,2'-diphenyl-1-picrylhydrazyl) initially exhibits an intense purple coloration which disappears and becomes colorless on contact with an antioxidant capable of saturating its electronic layer according to the following reaction :

DPPH. + AH → DPPH-H + A.

The decrease in the intensity of this coloration is detected by UV-visible spectrophotometry at 517 nm (Boulaaba et al. 2019). The reduction of DPPH highlights the anti-free radical power of the plant extract. After measuring the optical density, the results are expressed as a percentage inhibition and (IC_{50}) which is the concentration corresponding to 50% inhibition of the radical. The lower IC_{50} corresponds to the higher efficacy of the extract.

2.5.2. Iron Reducing Power

The reducing activity of iron is based on the redox reaction between the extract used at different concentrations and the transition metal ions, in particular iron or copper **[8].** In fact, the potassium III ferricyanide ($K3Fe(CN)_6$) used at 2.5 ml (1%) provides these $Fe3^+$ ions which will be reduced by the capacity of the antioxidants in the extract to give up electrons. The absorbance was measured at 700 nm. The results are expressed as an effective concentration (EC_{50}, µg / ml), which is the concentration of the extract corresponding to an absorbance equal to 0.5. The EC_{50} value is obtained by interpretation of the linear regression curve.

2.5.3. Total Antioxidant Capacity

Antioxidant activity is based on determining a concentration that confers efficacy on a given extract. This quantitative method consists in reducing the molybdenum (VI) or Mo6 + ions to molybdenum (V) or $Mo5^+$ by the extracts of the plant as well as the formation of the complex (phosphate / $Mo5^+$) of green color at an acidic pH **[4]** (**Zar Kalai et al. 2013**). This complex is revealed by the mixture of extract, sulfuric acid (0.6 N), sodium phosphate (28 mM) and ammonium molybdate (4 mM). Absorbance is measured at 695 nm against a blank containing methanol instead of the extract. As with total polyphenols, total antioxidant activity is expressed in mg of gallic acid equivalent per gram of dry matter (mg EAG / g DM).

2.5.4. Ability To Inhibit B-Carotene Bleaching

In the absence of antioxidants, the *β*-carotene solution undergoes rapid discoloration in the presence of fatty acids or lipids resulting from the breakdown of linoleic acid. However, in their presence, like phenolic compounds, the destruction of β-carotene is limited by the neutralization of radicals formed by the β-carotene-linoleate system **[4]** (**Zar Kalai et al. 2013**). Thus, a solution of β-carotene (2 mg) prepared with chloroform (20 ml) is prepared beforehand before being emulsified in hydrogen peroxide. In a 96-well microplate, 0.150 ml of this emulsion are added to 0.010 ml of plant extract of known concentration. The effect of the latter is estimated at 470 nm using a microplate reader (model EAR 400, Labsystems Multiskan MS). The activity of the extract is calculated relative to that of the positive control, 3-tert-butyl-4-hydroxyanisole (BHA) and to that of the negative control (without extract). This activity is also expressed as IC_{50} which is the concentration corresponding to 50% of the discoloration by the extract. The lower IC_{50} value corresponds to the efficacy of the higher extract.

3. Results and Discussion

3.1. Metabolites Contents and Antioxidant Activities

It is proposed, from the extract of the dry leaves of *Nitraria retusa* previously prepared with 70% ethanol, to measure the contents of total polyphenols, flavonoids and condensed tannins. The

phytochemical aspect was also analyzed and the measurements of some antioxidant activities such as the anti-radical capacity against DPPH, the reducing power of iron, the inhibitory effect of β-carotene bleaching and the total antioxidant capacity were carried out.

The results of the quantification of the phenolic compounds are presented in figure 1. The experiment results showed a richness of the leaves in these three secondary metabolites with values equal to 48.61 mg EAG / g DM for the total polyphenols, 50.53 and 2.07 mg EC / g DM, respectively for flavonoids and condensed tannins.

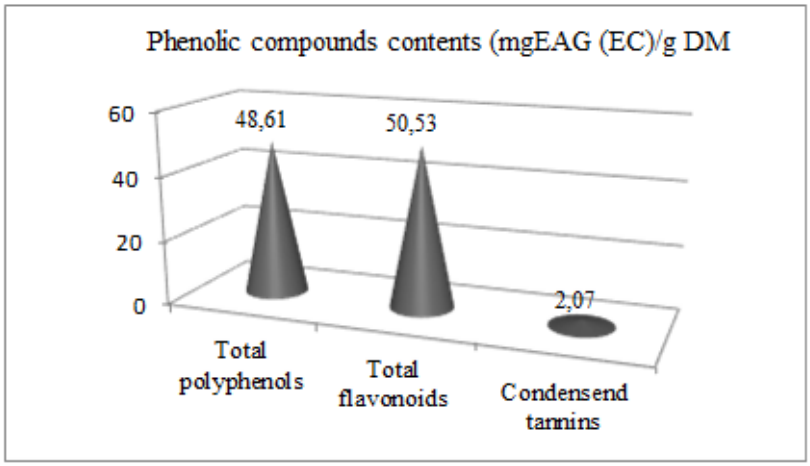

Figure 1. Contents of total polyphenols, flavonoids and condensed tannins relating to extracts of dry leaves of *N. retusa* expressed in mg EAG / g DM for the first group and in mg EC / g DM for the second and third group respectively.

N. retusa was collected during the summer (August) when temperatures are very high in addition to the high salinity of the soil in the Kairouan region (see materials and methods). In order to give an idea about these edaphic characteristics, the pH and the electrical conductivity (EC) were measured. The values are equal to 8.13 and 25.7 mS / cm respectively for pH and EC. For comparison, seawater has an EC of 56 mS / cm and has a pH of 8.2. Several studies about secondary metabolites -called stress metabolites relating to halophytes from this region of Kairouan have been published, namely *Limonium densiflorum* **[15]**, *Arthrocnemum indicum* **[7].** Thus, *N. retusa* produces more total polyphenols 48.61 (mg GAE / g DW) than *A. indicum* (19 mg GAE / g DW). Under the same environmental conditions, *N. retusa* shows higher levels of phenolic compounds than those of the halophyte *Suaeda fruticosa* **[10]**. In addition, a mixture of water and ethanol was used in this study to increase the chances of extracting phenolic compounds due to their polar nature. The results showed that the extract from *N. retusa* with 70% ethanol contains as many total polyphenols (48.61 mg EAG / g DM) as that of *L. densiflorum* for the same period and with 95 ethanol. % (48.04 mg EAG / g MS) **[15].**. The difference between the content of flavonoids and tannins can be a characteristic of the plant. On the other hand, the foliar extract of *N. retusa* has an important antioxidant power, first against the DPPH radical (63 µg / ml), then as a reducer of trivalent iron (0.92 mg / ml), then as an inhibitor of bleaching of β-carotene (59% for an extract used at 1 mg / ml) and finally capable of reducing molybdenum (VI) ions to molybdenum (V) (166.11 mg EAG / g MS) (Table 1).

Several studies have highlighted the direct and / or indirect relationship between the richness of extracts in phenolic compounds and the antioxidant activities of plants from arid regions **[3,4,7,8].** Antioxidants are essential to counter the effect of abiotic constraints (salinity and / or drought). The latter, at the origin of oxidative stress, are known to be responsible for the production of free radicals and cell vulnerability through alteration of DNA causing cell dysfunction or even death **[1].**

To show the reason why these bioactive molecules are synthesized, it was proposed to analyze the deterioration of the cell membrane of the leaves of *N. retusa* through the measurement of malondialdehyde (MDA) produced by the peroxidation of membrane lipids and the percentage electrolyte leakage (**Table 2**). In addition, measuring the relative loss of electrolytes (solutes) as charged cellular molecules that can conduct electricity is a widely used test to assess cellular damage caused by

oxidative stress **[5,3].** Indeed, the destruction of cell membranes induced by an abiotic stress such as salt, induces release of molecules called electrolyte leakage. A high value of the electrolyte leakage percentage indicates a strong leakage of ions through the membranes and therefore unstable membranes. In our present work, the MDA content relating to the leaf extracts is equal to 265.55 nmol / gMF and the percentage of electrolyte leakage is 65.4%. These measurements show that there has indeed been a degradation of membrane fatty acids. Moreover, on previous work on the relationship between salt and the reaction of the leaves of *Mesembryanthemum edule* treated at doses up to 600 mM NaCl, the authors noted on different provenances of *Mesembryanthemum* the percentages of leaks of electrolytes ranging from 34.7% to 61.4% and MDA contents not exceeding 5.24 nmol / g MF **[3].** Our results clearly show the impact of salt on *N. retusa* and the latter's cellular response. The levels of phenolic compounds are thus well explained.

In this study we also studied the contents of chlorophylls, carotenoids (light collectors, liposoluble and located in the chromoplasts), anthocyanins (water-soluble pigments in vacuoles) as well as an important solute, proline, as a stress indicators involved in the adaptation of extremophile plants (**Table 2**). Indeed, their quantifications provide information on the state of tolerance of halophytes. As an example of an oxygen reaction mechanism, the production of leaf radicals such as superoxide (O2−) is produced following a transfer of electrons at the level of the respiratory systems PSI and PSII of the chloroplast by reduction of oxygen O2 and using chlorophyll **[2].** Moreover, this pigment has long been measured in plants with various techniques including digital technology **[16].** This pigment is produced in small quantities when the photosynthesis process is disturbed. The work on *N. retusa* showed chlorophyll a and b contents equal to 6.16 (Chla) and 3.08 (Chlb) mg / ml. Carotenoids and anthocyanins are pigments that play a role in photosynthesis. When this is disturbed the contents of these molecules are affected. In the leaves of *N. retusa*, their contents are equal to 2.05 mg / ml and 0.145 µg / ml respectively. Carotenoids are very useful as regulators of homeostasis in so much drought **[17].** These pigments also have a chelating power of singlet oxygen 1O2 observed with the genus Arabidopsis, which surely explains the inhibitory effect of β-carotene bleaching **[18]**. On the other hand, the anthocyanins in the fruit juice of Nitraria tangutorum have a cardioprotective effect associated with antioxidant effects relating to the extract rich in these pigments **[19].** Proline also has a role in osmotic adjustment in a salt-rich environment and the results of this present work show values equal to 5.68 mg / g MF. As N. retusa was collected from a saline area, the production of this amino acid in the leaves is thus justified. Indeed, its intervention seems common to this kind of situation **[3,5].**.

Considering the antioxidant potential of the extract of *N. retusa*, it was proposed to determine the phytochemical profile of its leaf extracts by high performance liquid chromatography (HPLC).

Table 1. Overview of some antioxidant activities such as the anti-free radical capacity against DPPH (µg / ml), the reducing power of trivalent iron (mg / ml), the *β*-carotene bleaching test (µg /ml) and the total antioxidant capacity (mg EAG / g DM).

DPPH test (µg/ml)	RP test (mg/ml)	*β*-carotene test (µg /ml)	TAC (mg EAG/g DM)
63	0,92	59	166,11

Table 2. Measurements of malondialdehyde (MDA), percentage of leakage of electrolytes (EL), photosynthetic pigments chlorophyll a (Chla) and b (Chlb), carotenoids (Car), anthocyanins (Anth), as well as proline from leaves *of N. retusa*. Each measurement was repeated in triplicate.

MDA (nmol/g MF)	EL (%)	Chla (mg/ml)	Chlb (mg/ml)	Car (mg/ml)	Anth (µg/ml)	Proline (mg/g MF)
25,55±0,82	65,4±0,7	6,16±0,73	3,08±0,33	2,05±0,47	0,145±0,02	5,68±0,17

Table 3. Identification by HPLC at 280 (± 4 nm) of the phenolic compounds synthesized in the leaves of *N. retusa*.

N°	Retention time (mn)	Area	Area (%)	Identification
1	13,176	125,153	1,769	Catechol
2	14,507	52,606	0,743	Catechin
3	27,704	262,998	3,718	Hydroxycinnamic Acid
4	31,372	580,711	8,210	Hyperoside
5	34,986	768,507	10,865	Isorhamnetin-3-*O*-rutinoside
6	35,342	311,695	4,406	Isorhamnetin-3-*O*-glucoside
7	38,456	516,557	7,303	Trans-cinnamic Acid

3.2. Phytochemical Analysis by High Performance Phase-Liquid Chromatography (HPLC)

Based on the levels of phenolic compounds and the importance of antioxidant activities, it was proposed to investigate the phytochemical profile of the leaves of *N. retusa*. The phytochemical analysis of the leaf extracts confirms the existence of several phenolic compounds such as benzoic compounds catechol, flavonoids such as catechin, hyperoside (a glycoside of quercetin), isorhamnetin-3-o-rutinoside, isorhamnetin-3-o-glucoside as well as derivatives and precursors of phenolic acids such as hydroxycinnamic (also called coumaric acid) and trans-cinnamic acids (cinnamic acid is a precursor of many flavonoids and phenolic acids such as gallic acid) (**Table 3; Figure 3**). The chromatogram shows the existence of other unidentified compounds (**Figure 4**).

Various plants produce these molecules **[20,21,22,23,24,25,26]**, including halophytes [**4,7,27,8].** More specifically, isorhamnetin like kaempferol, catechin and even cinnamic acid or their derivatives are very frequently found in plants **[21,14,4,8].** The antioxidant power of the leaf extract of *N. retusa* seems due to the existence of the phenolic compounds produced by this plant. Indeed, several of these molecules (or their derivatives) detected or measured (such as anthocyanins) in leaves are known for their antioxidant powers such as catechin **[27]**, hyperoside **[28]**,cinnamic acid and its derivatives as well as anthocyanins and hydroxycinnamic acid **[20]** and isorhamnetin and its glycoside groups **[13].**

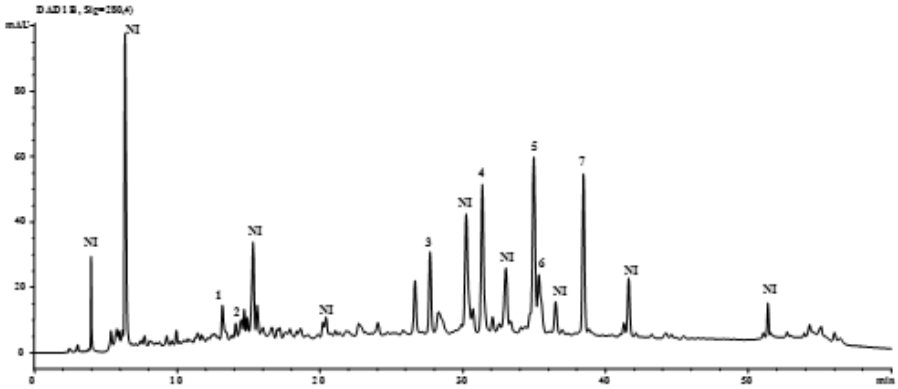

Figure 2. HPLC / UV chromatogram (280 ± 4 nm) for each molecule from 70% ethanol extracts of *Nitraria retusa* leaves. The compounds were identified by comparison of their retention time (RT) with those obtained from standard compounds. Peak identification : 1, catechol (RT : 13.176) ; 2, catechin (RT : 14.507) ; 3, hydroxycinnamic acid (RT : 27.704) ; 4, hyperoside (RT : 31.372) ; 5, Isorhamnetin-3-o-rutinoside (RT : 34.986) ; 6, isorhamnetin-3-o-glucoside (RT : 35.342) ; 7, trans-cinnamic acid (RT : 38.456).

4. CONCLUSION

The abiotic constraints are very diverse and under these conditions, physiological transformations occur characterized for example by a photosynthetic disturbance at the origin of the formation of reactive oxygen species. The latter are, among other things, at the origin of membrane disturbances such as lipid peroxidation. Various molecules are thus produced as stress indicators (osmolytes) associated with a synthesis of phenolic compounds with high antioxidant power. This is the case of *Nitraria retusa* which may could be a candidate for future research on the mechanisms of action of its biological effects.

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