EVALUATION OF PHENOLIC CONTENTS (QUANTITATIVE AND QUALITATIVE) AND ANTIOXIDANT ACTIVITIES IN DIFFERENT PHYSIOLOGICAL PHASES OF *Genista saharae* COSS. & DUR. GROWING IN THE SAHARA OF ALGERIA

Atef CHOUIKH[*], Fatma ALIA[*], Souad NEFFAR[**], Abdelkrim REBIAI[***], El Hadda ADJAL[*], Azzedine CHEFROUR[****]

[*] *Biology Department, Faculty of Natural Science and Life, El Oued University, Algeria*
[**] *Natural and Life Sciences Department, Tebessa University, Algeria*
[***] *Laboratory Valorization and Technology of Saharan Resources, El Oued University, Algeria*
[****] *Laboratory Development and Control of Hospital Pharmaceutical Preparations. Annaba University, Algeria.*
[*****] *Biology Department, Souk Ahras University, Algeria*
Corresponding author: Atef Chouikh, Biology Department, Faculty of Natural Science and Life, El Oued University, BP 789 El-Oued (39000), Algeria. Phone: 00213 666684715, e-mail: atef-chouikh@univ-eloued.dz / atchouikh@yahoo.fr

Abstract. To evaluate the natural products in the desert plants, antioxidant activity and phenolic contents of *Genista saharae* (Fabaceae) growing in Oued Souf region (Algerian desert) were determined during different phases of its development.

The results of the yield of methanolic extracts showed that the highest value was recorded in the extracts of the floral phase (8.6%) and the lowest was in the extracts of the fruiting phase (5.16 %). As we observed the relationship in the quantitative content of polyphenols and flavonoids, were recorded with the highest value of polyphenols at the first vegetative phase estimated as 1.33±0.018mg EAG/g Ex and 0.772 ±0.088mg EQu/g Ex, and the lowest value was in fruiting phase 0.443±0.078mg EAG/g Ex and 0.21±0.036mg EQu/g Ex.

While the results of antioxidant activity in the free radical revealed that, the extract of the second vegetative phase exceeded with a best inertial capacity than the other extracts (IC_{50}= 0.247 mg/ml). As for concerning the hemolysis test, the finding indicated at distinction of extract the first vegetative phase with a lower hemolysis 31.26%.

Using the HPLC, the qualitative analysis of extracts showed quantitative and qualitative differences concerning the presence of some phenolic compound such as: Gallic acid, Caffeic acid, ρ-Coumaric acid, Vanillin and Rutin in four extracts.

According to results obtained it seemed that there is a disparity in the phenolic content in terms of the different stages of growth. This seemed obvious in antioxidant activity results that can be the change in the quality and concentration of the phenolic compounds between different stages of growth.

Keywords: *Genista saharae*; Polyphenols; Flavonoids; Antioxidant activity; test DPPH; Hemolysis test; HPLC.

INTRODUCTION

In most often the oxidative stress refers to an imbalance between the production of free radicals and the antioxidant system [16, 31]. This attends the want for antioxidant agents who can arrest oxidative stress [3, 34]. A benefit of antioxidant phenolic compounds from trophic or food addition sources in the protection of inveterate malady have been reported in a large number of studies [1, 36]. Some plants are known to be a source of chemical compounds which are characterized by antioxidant properties [14]. These antioxidant compounds could possess the capacity to protect the cellular organelles from damage caused by free radicals induced oxidative stress [33]. Saharan plants are known by their resistance to several stress factors [7]. The *Genista* genus, from the family of Fabaceae, consists in 25 species, mainly represented in Algeria among them 11 species are endemic [35]. *Genista saharea* is an endemic Saharan medicinal sapling [15], localized in North Africa (Algeria, Libya, Morocco, Tunisia and Egypt) [24, 29, 35], used in traditional pharmacopoeia by topical population against sundry diseases as an herbaceous remedy for infections of the respiratory system [7]. It is also used for feeding animals [29]. Effectively, it is frequently appreciated by camels because of its serious urine retention capacity in this livestock. In addition to their pastoral qualities, it is favorable for sand stabilization and combating desertification [15].

Some previous studies have evaluated the chemical composition and antioxidant activities of this genus [28]. Unfortunately, to date, there are still no biological data affordable about this endemic Algerian shrub, especially growing in arid region of Oued Souf. Also, there is no literature report about the antioxidant activity for its alcoholic extract. Consequently, this work aims at monitoring the phenolic content quantitative and qualitative between different stages of growth and the relationship of this and antioxidant activity of *Genista saharae* (Fabaceae).

MATERIALS AND METHODS

Plant material

Different samples of the aerial partof plant were collected at the region of Ghammra (Oued Souf, Algeria) located at a longitude (06°48’08.3” East) and latitude (33°33’10.2” North).

The plant was harvested during different stages of growth (Table 1). All samples were dried at room temperature, protected from light. After crushed samples are put in papery sacs; than submitted to extraction. The biological tests were transacted in laboratory of the University of Echahid Hamma Lakhdar – El-Oued- Algeria.

Chemicals

Methanol 99% (MeOH), distilled water, Gallic acid, sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, Quercetin, Aluminum Chloride ($AlCl_3$),

Table 1. Date of the collection to different stages of growth of plant.

Developmental stage	Date of the collection
The first vegetative phase	11 March 2016
The floral phase	26 March 2016
The fruiting phase	01 June 2016
The second vegetative phase	19 September 2016

Ascorbic acid; 1,1-dipheny-2-picryl-hydrazil (DPPH$^{\bullet}$), Ferric Chloride ($FeCl_3$), Peroxide(H_2O_2).

Extraction procedure

Fifty grams (50g) of every sample material were macerated with 250 ml of methanol (for best qualitative and quantitative of compounds) at room temperature in dark for 24h (repeated 3 times). The solvent was filtered by papers of Watman, evaporated and concentrated under rotary vapor at 50° C.

The extraction yield is presented in percentage (%) by formula given by [15].

$$\%Yield = (M_E/M_S) \times 100$$

Where in: $\mathbf{M_E}$ is the mass after evaporation of solvent in g; $\mathbf{M_S}$ is dry mass of the sample in g.

Total phenolic content

Total phenolic content in the four samples was determined by the Folin Ciocalteu reagent [42] according to the method of [6] with slight modifications. Into test tubes, 0.2 ml of the different concentrations of sample was mixed with 1 ml of Folin-Ciocalteu reagent (10%). After, several minutes we has added 0.8 ml of Na_2CO_3 solution (7.5%).The reaction was incubated at room temperature in the dark for 30 min. After this, the absorbance was read at λ= 765 nm by UV-Vis spectrophotometer. Some different concentrations (0.02-0.12 mg/ml) of Gallic acid was used for the standard calibration curve. The results were expressed as mg of Gallic acid equivalent per g (mg GA E/g Ex).

Total flavonoids content

The flavonoids content was determined in colorimetric method through using the Aluminum chloride by method cited by [10], which 0.5 ml of the different concentration of sample was added to 0.5 ml of solution $AlCl_3$ (2%). After incubation for 1h at room temperature in the dark, the absorbance was measured at λ= 420 nm by UV-Vis spectrophotometer. Quercetin (0.1-0.3 mg/ml) was used as a standard. The results were expressed as mg of Quercetin equivalent per g of extract (mg Qu E/g Ex).

Antioxidant activities

The antioxidant activity of every sample evaluating by two tests: test of the free radical DPPH and the test of hemolysis.

DPPH$^{\bullet}$ radical scavenging test

In the test we were based on the method cited by [8] with some modifications. Briefly, 0.5 ml of each sample at different concentration was mixed with 1ml (0.1 mM) of DPPH$^{\bullet}$ solution. The mixture shake well, after 15 min at room temperature in the dark, the absorbance was measured at λ= 517 nm by UV-Vis spectrophotometer [37]. The different concentrations of Ascorbic acid were prepared for used a reference standard (positive control at the extracts). The percentage scavenging of DPPH$^{\bullet}$ radical was measured by using the following equation:

$$\%\ Inhibition = [(A_C - A_S) - A_C] \times 100$$

where in: $\mathbf{A_C}$: the absorbance of the DPPH solution (the control reaction); $\mathbf{A_S}$: the absorbance of mixture of extracts or standard and DPPH solution.

The inhibition of 50% of concentration of DPPH$^{\bullet}$ radical free (IC_{50}) of the *Genista saharae* extracts is calculated from equation linear of the percentage inhibition versus concentration of inhibition. It was expressed as mg/ml.

Hemolysis test

The objective of this test to determine the protection of the erythrocytes blood cell from the explosion by our extracts, after exposure to oxidative stress by measuring the proportion of degenerated erythrocytes [12]. Hemolysis was assayed by measuring the concentration of liberate serum hemoglobin [23].

Sample of blood was obtained from healthy human (O^+) by method of the preparation of [25] with little modification. The experimental protocol was based on the method used by [2], with some modifications. Briefly, 40 µl from erythrocytes of blood was shuffled for 2 ml of each sample at different concentration of the extract, after this solution was conserved 5 min in 37° C of temperature. Another, 40 µl from H_2O_2 (30 ml mol) and $FeCl_3$ (80 ml mol) were added to the jumbles. After 1h of incubation at 37° C, the mix were centrifuged with 700 Tour / min to 10 min, the absorbance of supernatant was obtained as λ= 540 nm by UV-Vis spectrophotometer [2]. Ascorbic acid was used as a reference standard (positive control). The percentage of hemolysis was determined using the following equation:

$$\%\ Hemolysis = (A_C - A_S) \times 100$$

where in: $\mathbf{A_C}$ is the absorbance in the absence of the extracts; $\mathbf{A_S}$ is the absorbance in the presence of the extracts or standard.

HPLC analysis

For detection of phenolic compounds we use of High Performance Liquid Chromatography (HPLC), which is the most used technique for separation of phenolic compounds [20]. It permits simultaneous separation, identification and quantification of phenolic compounds in a short time [44]. The principle of HPLC separation is based on the distribution of analyses between stationary and mobile phase. In this case, stationary phase contains linked non-polar aliphatic residues and mobile phase in made up of polar solvents (acetic acid, distilled water, perchloric acid or formic acid in an organic solvent such as methanol or

acetonitrile) [18]. Ultraviolet/visible, photodiode array and UV-fluorescent detectors are commonly used for detection of phenolic compounds [22]. Briefly, according to [33] with few modifications; the effluent was detected at λ = 268 nm, 20µl of plant extract solution(between 1-5 mg/ml) is injected into the injector flow stream, next it adjusts the high pressure that drives the phase mobile solvent in the column (phase stationary), the separation of the compounds in mixture based by using its molecular weight and polarity, the compounds determined by the detector connected to the column on the one hand and by the computer on the other hand. The results reserved in form of chromatographs characteristic with the peaks.

Statistical analysis

Each value was the mean ± SD of three experiments. The correlation between these mean values was performed by Microsoft Excel 2010.

RESULTS

Yield of extracts

The yield of extracts of aerial parts of different samples of *G. saharea* (Fig. 1) was locate the maximal in the extract of the flowering phase to be 8.6 ± 0.12%, and the minimal was in the extract of the fruiting phase to be 5.16 ± 0.1 %. Whilst, the rapprochement at the results were marked between the extract of the first vegetative phase and the extract of the second vegetative phase to be 7.08 ± 0.08 % and 7.44 ± 0.15%, respectively.

Total phenolic and flavonoids content

The total phenolic and flavonoids of methanolic extracts from *G. saharae* is presented in (Fig. 2). We observed the relationship in the quantitative content of the polyphenols and the flavonoids. Whereat the total phenolic content varies from 1.33±0.018 to 0.443±0.078 mg EGA/g Ex, and flavonoids content from 0.772 ±0.088 to 0.21±0.036 mg EQu/g Ex. The highest amounts in this plant for both were marked with the first vegetative phase, while, the less values were showed in fruiting phase.

Antioxidant Activity

DPPH• Radical scavenging

The results of radical scavenging activity of extracts and standard (ascorbic acid) were presented in (Fig. 3) We observed the value of IC_{50} of ascorbic acid (IC_{50}= 0.015 mg/ml) superior on other values of the extracts of plant. Also, the extract of second vegetative phase marked best value of (IC_{50}= 0.247 mg/ml) in comparison with other extracts. Generally, the results indicate that extracts of *G. saharae* has a reduced potential with radical scavenging activity.

Hemolytic assay

According to results described in (Fig. 4) we observed the inverse relation between the percentage of hemolysis and the various extracts of plant *G. saharae.* In this assay, the percentage of hemolytic ranging from 9.07 % to 34.81%, where we was found at distinction of the ascorbic acid on the all extracts plant, among the extracts the lower anti-hemolysis activity is noteworthy that the extract of the first vegetative phase to be 31.26 % at C =1.2 mg/ml.

In this study, the antiradical activity of extracts was found significantly. A correlation (Fig. 5) was established between the phenolic content and antiradical activity (R^2 = 0.9446), and between the phenolic content and the percentage of hemolytic after

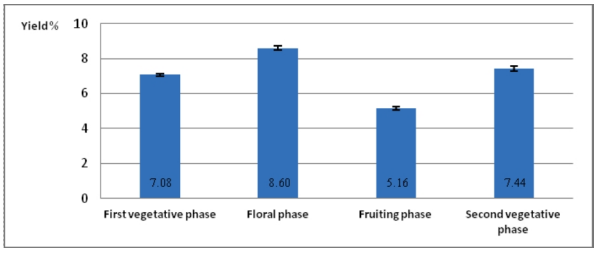

Figure 1. The yield (%) of different extracts methanolic of *G. saharae*

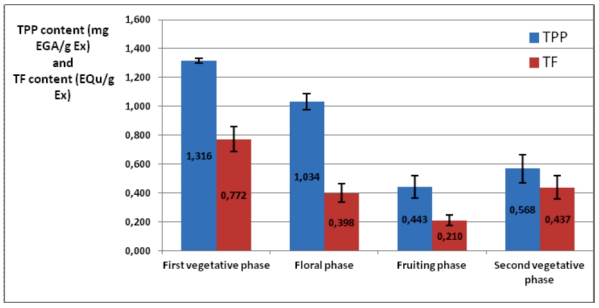

Figure 2. Total polyphenol and flavonoids contents of each extract of *G. saharae*

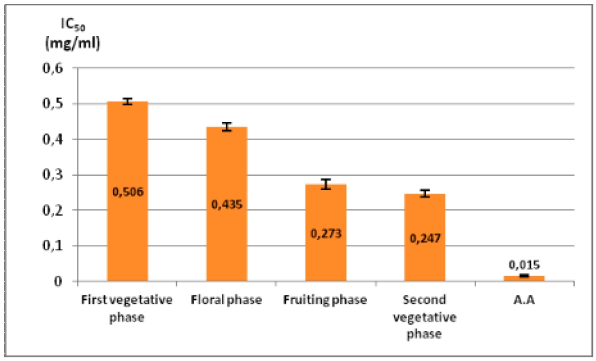

Figure 3. The IC_{50} values (DPPH• test) of the extracts of *G. saharae* and ascorbic acid

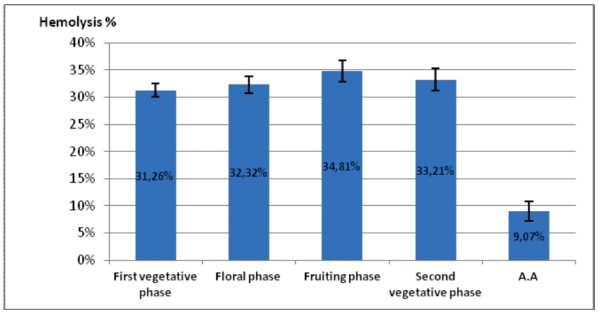

Figure 4. Rate of Hemolysis after use the extracts of *G. saharae* and ascorbic acid with [C] = 1.2 mg/ml

use of extracts (R^2 = 0.9093). But the anti-radicals activities linked to the structure and quality of phenolic compounds.

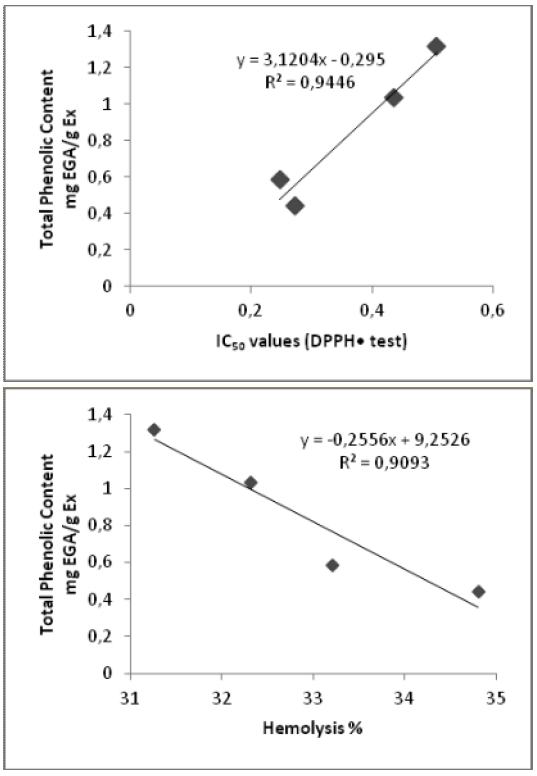

Figure 5. the correlation between phenolic content and antioxidants activities of the extracts of *G. saharae*

HPLC analysis

The chromatograms in the (Fig. 6) showed the phenolics compounds at extracts of *G. saharea*. These results indicated the difference of numbers of phenolic compounds for every extract, which ranged from 37 to 52 compounds.

Also, it is known that the best known phenolic compounds are obtained, such as: gallic acid, caffeic acid, vanillin, ρ-Coumaric acid and rutin.

The gallic acid and ρ-Coumaric acid were exhibited with all extracts studied, whilst, the rutin was vanished in the extract of the fruiting phase as such is shown in (Table 2). On the other hand, these results explained the contrast of the concentration for phenolic compounds as it is pronounced in (Table 2).

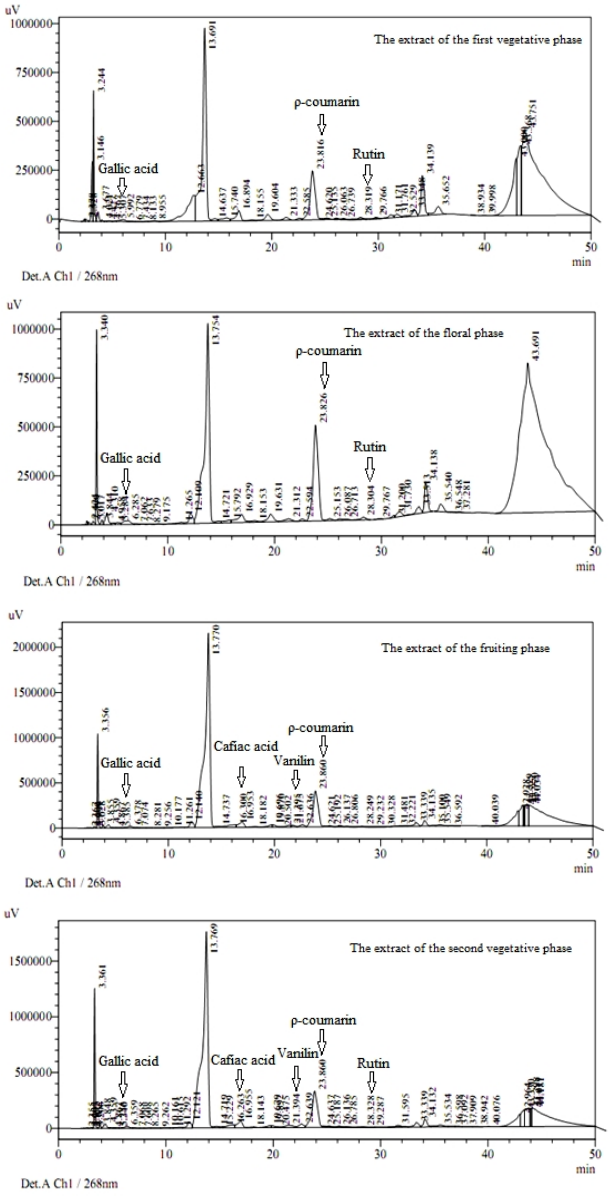

Figure 6. HPLC chromatogram's of extracts of *G. saharae*

After examined the correlations between the phenolic compounds and the tests antioxidants we found only two correlations (Fig. 7). The first highly correlation is between IC_{50} and caffeic acid (R^2 = 0.8778); the second one is medium value between IC_{50} and Rutin (R^2 = 0.6042).

Table 2. The concentration (µg/mg Ex) for some phenolic compounds in the different extracts of *G. saharae*

Extracts / Phenolic Compounds	First vegetative phase	Floral phase	Fruiting phase	Second vegetative phase
Gallic acid	0.210	1.032	0.518	0.317
Caffeic acid	/	/	4.086	3.127
ρ-Coumaric acid	32.83	65.76	52.34	50.36
Vanillin	/	/	2.104	3.903
Rutin	2.524	3.398	/	1.368

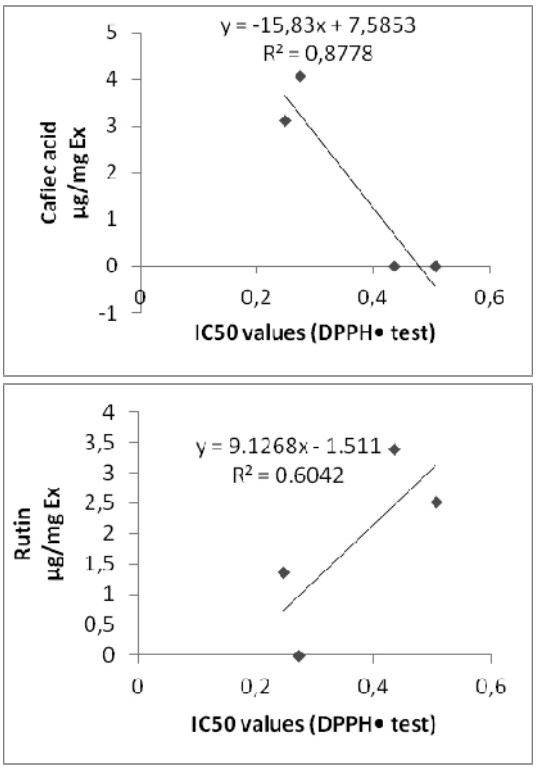

Figure 7. the correlation between IC_{50} and some phenolic compounds in the different extracts of *G. saharae.*

DISCUSSION

Extraction yield

The yields for different samples of aerial parts of *G. saharae* were obtained by maceration which was variable, where we can suggest the cause in yield for the nature of chemical compounds [41], the complexity and length of carbon chains [21], the physiological activity and the status of plants at different age levels [43], in addition to this, the reason may be due to the exposure of the plant to different stresses [13].

Total phenolic and flavonoids content

In the present study were found the gradual reduction of the quantitative content of total polyphenols and flavonoids in the three principal phases at age of the plant; which is usually accompanied by physiological changes [4, 29]. Moreover; the some phenolic compounds can be modify into other compounds such as pigmentation [32], saccharides [1] and volatility compounds [21].

According to [22] the total content of polyphenols and flavonoids varies from one species botanic to other and from stage physiologic to another; whilst [41] pointed out existence of motion to inhibit the genes expressed by the enzymes, whereas the result of the second vegetative stage in both the total polyphenols and flavonoids can be explained by the plant's restoration of its bioactivity a causing from release to new life cycle [38].

DPPH• Radical scavenging

The results obtained in our study indicates that the extract was exhibited feeble of radical scavenging activity. It can be explained by the few quantity of the total polyphenols contents, where the previous studies indicated until the phenolic compounds are good substances for the antioxidant activity due to the presence of numerous hydroxyl groups, which can react with free radicals [19]; in addition to the binary liaison at chemical structures between C2-C3 and in OX-4 groups [9] and the relationship between nature, structure and concentration of phenolic contents and antiradical scavenging activity [27].

Hemolytic assay

Erythrocytes are considered as major targets of free radicals [2] owing to the presence of high concentration of polyunsaturated of fatty acids in membrane [5, 11] and the oxygen transport by redox active hemoglobin molecules [2]. The hydroxyl radicals are the major active oxygen species causing of enormous biological damage trough lipid peroxidation [23]. The result indicated in this research showed the capacity acceptable of the extracts for the protection anti-hemolysis. According to previous studies we can explain these results by quality and functional capability of the total phenolic contents protect of the cellular membrane from oxidation.

In this context the phenolic compounds exert their protective effects through diverse mechanisms such as: blocking or suppressing agents oxidative or by activating a signaling cascade that activates detoxifying enzymes for elimination of ROS or inducing apoptosis [26].

HPLC analysis

The qualitative analysis of methanolic extracts of *G. saharae* by HPLC was confirmed the presence of some phenolic compounds such as Gallic acid, Caffeic acid, Vanillin, ρ-Coumaric acid and Rutin. These compositions were found in different concentrations between various stages of plant; consequently, this result can be explained by the physiological role of every compound during the different stages of growth, for gallic acid was been helped to adaptation the plans with the climatic conditions and it has been reported as one of the compounds responsible for the allelopathic effects between the plants [45]. The Caffeic acid was been accelerated to aging of plants [40] thence we were suggested the cause of his apparition in the fruiting phase and the backed in the second vegetative stage, adding to absence in whoever the first vegetative and the floral phase.

The vanillin does accelerate the maturity of fruits by modifying the taste and flavor of the fruit [30] and it has the role at anti-abiotic stress [17]. As for ρ-Coumaric acid has a role in the reduction of the vegetative growth of the plants and it is a good stimulated for antioxidant activity [40], so we can explain our results at this cause.

Concerning the rutin it plays an important role in organizing the hormones of vegetative growth [39].

REFERENCES

[1] Aberoumand, A., Deokule, S.S., (2008): Comparison of phenolic compounds of some edible plants of Iran and India. Pakistan Journal of Nutrition, 7(4): 582-585.

[2] Abirami, A., Gunasekaran, N., Perumal, S., (2014): *In vitro* antioxidant, anti-diabetic, cholinesterase and tyrosine inhibitiry potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. Food Science and Human Wellness, 3(1): 18-22.

[3] Atmani, D., Chaher, N., Berboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., Debbache, N., Atmani, D., (2009): Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chemistry, 112(2): 303-309.

[4] Bacelar, E., Santos, D., Moutinho-Pereira, J., Bert, G., Ferreira, H., Correia, C., (2006): Immediate responses and adaptive strategies of three olive cultivars under contrasting water availability regimes: Changes on structure and chemical composition of foliage and oxidative damage. Plant Science Journal, 170(3): 596-605.

[5] Banerjee, A., Kunwar, A., Mishra, B., Priyadarsini, K.I., (2008): Concentration dependent antioxidant/pro-oxidant activity of curcumin: Studies from AAPH induced hemolysis of RBCs. Chemico-Biological Interactions, 174(2): 134-139.

[6] Beretta, G., Granata, P., Ferrero, M., Orioli, M., Maffei Facino, R., (2005): Standardization of antioxidant properties of honey by a spectrophotometric/fluorimetric assay and chemo metrics. Analytica Chimica Acta, 533(2): 185-191.

[7] Bouchouka, E., Djilani, A., Bekkouche, A., (2012): Antibacterial and antioxidant activity of three endemic plants from Algerian Sahara. Acta Scientiarum Polonorum Technologia Alimentaria, 11(1): 61-65.

[8] Brand-Williams, W., Cuvelier, M.E., Berset, C., (1995): Use of free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1): 25-30.

[9] Cai, Y., Luo, Q., Sun, M., Corke, H., (2004): Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science, 74(17): 2157-2184.

[10] Chouikh, A., Adjal, E.H., Mekki, M., Hemmami, H., Feriani, A., Rebiai, A., Zaater, A., Chefrour, A., (2016): Comparison of ultra-sound and maceration extraction methods of phenolics contents and antioxidant activities of Saharian medicinal plant *Calligonum comosum* L'her. Journal of Materials and Environmental Science, 7(6): 2235-2239.

[11] Cooper, G., (1999): La cellule une approche moléculaire. 1ère Edition. Sinauer Associates, Massachusetts, 706 p.

[12] Dolci, A., Panteghini, M., (2014): Harmonization of automated hemolysis index assessment and use: Is it possible?. Clinica Chimica Acta, 432: 38-43.

[13] Ebrahimi Nejad, S., Hadian, J., Mirjalili, M.H., Sonboli, A., Yousefzadi, M., (2008): Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phonological stages. Food Chemistry, 110(4): 927-931.

[14] Gramza, A., Korczak, J., Amarowicz, R., (2005): Tea polyphenols - their antioxidant properties and biological activity - a review. Polish Journal of Food and Nutrition Sciences, 14(3): 219-235.

[15] Guettaf, S., Abidli, N., Kariche, S., Bellebcir, L., Bouriche, H., (2016): Phytochemical screening and antioxidant activity of aqueous extract of *Genista saharae* (Coss. & Dur.). Der Pharmacia Lettre, 8(1): 50-60.

[16] Han, X., SHEN, T., LOU, H., (2007): Dietary polyphenols and their biological significance. International Journal of Molecular Science, 8(9): 950-988.

[17] Inderjit, Dakshini, K.M.M.D, Chester, L.F., (1999): Principales and Practices in Plant Ecology: Alleochemical Interaction. CRC Press, Florida, 608 p.

[18] Jaclyn, S., El-Sayed, M.A., (2010): Food Applications and Physiological Effects of Anthocyanins as Functional Food Ingredients. The Open Food Science Journal, 4: 7-22.

[19] Javanmardi, J., Stushnoff, C., Locke, E., Vivanco, J.M., (2003): Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chemistry, 83(4): 547-550.

[20] Jiri, D., Ondrej, Z., Jiri, S., Branislav, R.N., Petr, B., Miroslava, B., Jindrich, K., Jaromir, H., Borivoj, K., Rene, K., Vojtech, A., (2013): Electrochemical tools for determination of phenolic compounds in plants. International Journal of Electrochemical Science, 8: 4520-4542.

[21] Khali, M., Mahmoudi, N., Mahmoudi, S., (2013): Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). Revue «Nature & Technologie» – Science Agronomique et Biologique, 9: 35-40.

[22] Klejdus, B., Lojková, L., Lapčík, O., Koblovská, R., Moravcová, J., Kubáň, V., (2005): Supercritical fluid extraction of isoflavones from biological samples with ultra-fast high-performance liquid chromatography/mass spectrometry. Journal of Separation Science, 28: 1334-1346.

[23] Lippi, G., Salvagno, G.L., Montagnana, M., Brocco, G., Guidi, G.C., (2006): Influence of hemolysis on routine clinical chemistry testing. Clinical Chemistry and Laboratory Medicine, 44(3): 311-316.

[24] Maire, R., (1987): La flore de l'Afrique du Nord. Editions Le chevalier, Paris, 302 p.

[25] Malagobi, D., (2007): A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. Invertebrate Survival Journal, 4(2): 92-94.

[26] Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., (2004): Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, 79(5): 727-747.

[27] Marius, L., Rakiatou, T., Noufou, O., Felix, K., Andre, T., Pierre, D., Pierre, G.I., (2016): *In vitro* antioxidant activity and phenolic contents of different fractions of ethanolic extract from *Khaya senegalensis* A. Juss. (Meliaceae) stem barks. African Journal of Pharmacy and Pharmacology, 10(23): 501-507.

[28] Mekiou, R., Touahr, H., Dijoux-Franca, M.G., Mariotte, A. M., Benayche, S., Benayche, F., (2005): A new Isoflavone from *Genista saharae*. Biochemical Systematics and Ecology, 33(6): 635-638.

[29] Meriane, D., Genta-Jouve, G., Kaabeche, M., Michel, S., Boutefnouchet, S., (2014): Rapid identification of antioxidant compounds of *Genista saharae* Coss. & Dur. by combination of DPPH scavenging assay and HPTL – MS. Molecules, 19(4): 4369-4379.

[30] Metzler, D., (2003): Biochemistry: the chemical reactions of living cells. 2nd edition. Acadimic Press Elsevier, Massachusetts, 1973 p.
[31] Mishra, K., Ojha, H., Chaudhury, N.K., (2012): Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry, 130: 1036-1043.
[32] Neto, L., Uchoa, A., Moura, A., Filho, B., Tenorio, G., Gomse, A., Ximenes, R., Vanusa, M., Correia, M.T., (2016): Phytochemical screening, total phenolic content and antioxidant activity of some plants from Brazilian flora. Journal of Medicinal Plants Research, 10(27): 409-416.
[33] Ojeil, A., El Darra, N., El Hajj, Y., Bou Mouncef, P., Rizk, J.T., Maroun, R.G, (2010): Identification et caracterisation de composes phenoliques extraits du raisin Chateau Ksara. Lebanese Science Journal, 11(2): 117-131.
[34] Pourmorad, F., Hosseinimerhr, S.J., Shahabimajd, N., (2006): Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology, 5(11): 1142-1145.
[35] Quezel, P., Santa, S., (1962): Nouvelle flore de l'Algérie et des régions désertiques mériditionneles. CNRS, Paris, pp. 470-475.
[36] Rajaei, A., Barzegar, M., Hamidi, Z., Sahari, A., (2010): Optimization of extraction condition of phenolic compounds from pistachio (*Pistacia vera*) green hull through response surface method. Journal of Agricultural Science and Technology, 12: 605-615.
[37] Rebiai, A., Lanze, T., Chouikh, A., (2015): Physicochemical and biochemical properties of honey bee products in south Algeria. Scientific Study & Research – Chemistry and Chemical Engineering, Biotechnology, Food Industry, 16(2): 133-142.
[38] Renard, C.M.G.C., Caris-Veyrat, C., Dufour, C., Le Bourvellec, C., (2014): Le devenir des polyphénols et caroténoïdes dans les fruits et légumes traités thermiquement. Innovations Agronomiques, 42: 125-137.
[39] Sajeeth, C.I., Manna, P.K., Manavalan, R., Jolly, C.I., (2010): Quantitative estimation of Gallic Acid, Rutin and Quercetin in certain herbal plants by hptlc method. Der Chemica Sinica, 1(2): 80-85.
[40] Schnablova, R., Synková, H., Vicanková, A., Burketová, L., Eder, J., Cvikrová, M., (2006): Transgenic ipt tobacco overproducing cytokinins overaccumulates phenolic compounds during in vitro growth. Plant Physiology and Biochemistry, 44(10): 526-534.
[41] Sideney, B.O., Dirceu, A., Amarildo, A. T., Alessandra, B.T., (2016): Total phenolic, flavonoid content and antioxidant activity of *Vitex megapotamic* (Spreg.) Moldenke. Ciência e Natura, 38(3): 1197-1204.
[42] Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol, 299: 152-178.
[43] Tlili, N., Mejri, H., Yahia, Y., Saadaoui, E., Rejeb, S., Khaldi, A., Nasri, N., (2014): Phytochemicals and antioxidant activities of Rhus tripartitum (Ucria) fruits depending on locality and different stages of maturity. Food Chemistry, 160: 98-103.
[44] Tomás-Barberán, F.A., Iniesta-Sanmartin, E., Ferreres, F., Tomás-Lorente, F., (1990): High Performance Liquid Chromatography, thin layer chromatography and ultra violet behavior of flavone aglycone with unsubstituted B rings. Phytochemical Analysis, 1(1): 44-47.
[45] Weidenhamer, J.D., Li, M., Allman, J., Bergosh, R.G., Posner, M., (2013): Evidence does not support a role for gallic acid in *Phragmites australis* invasion success. Journal of Chemical Ecology, 39(2): 323-332.

Received: 1 Septembre 2018
Accepted: 30 October 2018
Published Online: 8 November 2018
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House