EFFECT OF ENVIRONMENTAL FACTORS ON VARIATION OF PHENOLIC COMPOUNDS IN LEAVES OF *Pistacia lentiscus* L. AND *Pistacia atlantica* Desf.

Fatma CHEHRIT-HACID[*], Rabéa GACEB-TERRAK[**], Henia DAOUDI[***], Lynda HANNACHI[***], Boussad METNA[***], Abderrahmane MATI[*], Ferid LIMAM[****], Farida MATI-MOULTI[*]

[*] *Laboratory of Analytical Biochemistry and Biotechnologies, Faculty of Bioagronomy, M. Mammeri University, Tizi-Ouzou, Algeria*
[**] *Laboratory of Research on Arid Areas, Faculty of Biosciences, University of Sciences and Technology, Houari Boumediene (USTHB), BP 32, El-Alia 16111, Bab-Ezzouar, Algiers, Algeria*
[***] *Laboratory of Natural Resources, M. Mammeri University Tizi-Ouzou, Algeria*
[****] *Laboratory of Bioactive Substances, Center of Biotechnology of the Techno pole Borj-Cedria, BP. 901, 2050 Hammam-Lif, Tunisia.*
Correspondence author: Fatma Chehrit-Hacid, Laboratory of Analytical Biochemistry and Biotechnologies, Faculty of Bioagronomy, M. Mammeri University, Tizi-Ouzou, Algeria, phone: +2132618615, e-mail: hacidummto@yahoo.com

Abstract. *Pistacia lentiscus*, evergreen sclerophyll shrub and *P. atlantica*, deciduous species, belong to *Anacardiaceae* family. They were chosen in this study because they suit the Mediterranean climate characterized by hot and dry summers and mild winters with heavy rain. Polyphenols are involved in the response to biotic and abiotic stress. However, very few studies have been conducted on the polyphenols of *P. lentiscus* and *P. atlantica* and to our knowledge, there are no studies on phenolic variability. The aim of this work is to study polyphenolic variability in *Pistacia lentiscus* and *P. atlantica* leaves, in relation to environmental factors. The environmental factors taken into account are altitude, slope exposure and some soil characteristics as pH, organic matter and Calcium carbonate. The total polyphenols were extracted with 70% ethanolic solvent and phenolic aglycones with diethylether after acid hydrolysis. HPLC-DAD was used to identify and quantify polyphenolic compounds in the extracts when GC-MS was used to identify the aglycones. Total polyphenols, total flavonoids and hydrolysable tannins were quantified using an UV/Visible spectrophotometer. In order to understand the variability within and between the two species and its relation to certain environmental factors, principal compound analysis was performed. The results obtained showed the presence of high amounts of polyphenols in the leaves of both species, the total polyphenol content varies from 19.50 ± 2.5 $mg \cdot g^{-1}$ DM to 28.56 ± 3.61 $mg \cdot g^{-1}$ DM. They also show intra and inter specific variability with respect to the environmental factors. Presence of several chemotypes of the two species were suggested. *P. atlantica* was qualitatively and quantitatively richer and relatively more homogeneous than *P. lentiscus* wich presents high intraspecific variability, fourteen compounds were detected by HPLC-DAD and more than twenty five by CG-MS.

Key words: *Pistacia lentiscus*; *P. atlantica*; phenolic compounds; environmental factors.

INTRODUCTION

The Mediterranean climate is characterized by hot and dry summers and mild winters with abundant rains; which gives them great temporally and spatially variability [8, 14]. This climatic particularity, because of its location in a transition zone between the temperate western winds and the strong subtropical pressures [3, 37], makes Algeria, like the Mediterranean region, one of the areas most affected by climate change [39]. Thus, the crucial problem that arises with regard to the climatic and socio-economic challenges is the selection of appropriate species for land restoration. This choice must take into account natural and rustic vegetation that is dominated by sclerophyllous or malacophyllous woody shrubs and trees that can withstand long periods of dry summer [15]. In this context, our study focused on two species: *P. lentiscus*, evergreen sclerophyll shrub [28] and *P. atlantica*, deciduous species, belonging to the genus *Pistacia* [11, 32]. *P. atlantica* is distributed from the Canary Islands in North Africa to Iran in the Near East while *P. lentiscus* is found all around the Mediterranean both on the north shore and on the south shore [47].

Polyphenols are involved in the biochemical mechanisms of defense and control of various biotic and abiotic stresses [34]. Flavonoids, by their ability to neutralize reactive oxygen species, play an important role as photoprotective agents [1]. This characteristic linked to the physicochemical properties of polyphenols could be used as an indicator of the strength of the species. Several studies have shown that environmental factors can influence qualitatively and quantitatively the production of polyphenols [7, 21, 29]. Very few studies have been conducted on the polyphenols of *P. atlantica* and *P. lentiscus* [36, 45] and to our knowledge, there are no studies on their phenolic variability.

The objective of this work is the comparative study of the phenolic variability of *P. atlantica* and *P. lentiscus* leaves in relation to environmental factors as well as by the identification and quantification of different classes of phenolic compounds present in both species.

MATERIAL AND METHODS

Plant material

The leaves of *P. lentiscus* and *P. atlantica* were collected between June and August 2014 in six sites located in northern Algeria: Azeffoun, Ouezra and Tarik Ibn Ziyad for *P. lentiscus* and Thniet El Had (north slope of Tisemsilt), Sidi Boutouchent (south slope of Tisemsilt) and Ain Oussera for *P. atlantica*. The main geographical and climatic characteristics have been summarized in the table 1. Pedological feature of the sites are given in the table 2. The identification of species was made by Dr. M. Laribi (M. Mammeri University, Tizi-Ouzou, Algeria, Faculty

of Biological and Agronomical Sciences, Department of Biology), which is in perfect agreement with the description established by Quezel & Santa [31]. The voucher specimens (MP-1-16-12 for *P. lentiscus* and MP-1-16-1 for *P. atlantica*) were deposited at the Natural Resources Laboratory, M. Mammeri University, Tizi-Ouzou (Algeria). From each site, leafy twigs were individually collected from five trees. For each species, the leaves of the five samples were dried separately at room temperature and protected from humidity and bright light; they were then reduced to a fine powder, which is stored in sterile smoked glass bottles. Biochemical analyzes were carried out in 2015 in order to assess the possible influence of environmental factors (altitude, slope exposure, soil pH, organic matter and calcium carbonate) on the quality and quantity of phenolic compounds in the different populations of *Pistacia*.

Soil characteristics of study sites

Soil, an important component of environmental factors, exerts a strong selective pressure and a strong adaptation of plant species and consequently on the distribution of vegetation [33]. Indeed, soil provides plants with foothold for their roots and holds the necessary nutrients for plants to grow [16]. It is therefore important to characterize it in order to examine the possible influence on the phenolic characteristics of the different populations of *P. atlantica* and *P. lentiscus*. Approximately 500 g of soil samples were collected under each tree at the four cardinal points from 0 to 10 cm deep and mixed to form a composite sample at each site. They were used to measure characteristics given in table 2.

Extraction of polyphenol

Total polyphenol extraction

The efficiency of extraction of polyphenols depends on several parameters such as temperature, type of solvent, duration of extraction and the solid / solvent ratio [24]. Several solvents are usually used. The chemical nature of polyphenols also influences the extracton yield [13]. In our study we opted for a more ecological, simpler, faster and less expensive method [42], described by Mahmoudi et *al.* [25] and Mocan et *al*. [27] with some modifications. Thus, leaf samples (0.5 g) were extracted with 80% ethanol (5 mL) at 50°C for 30 min. The solutions were centrifuged at 2400g for 15 min; the recovered supernatants are adjusted to 80% with ethanol and stored at -20°C. They were used to quantification of total polyphenols (TPP), total flavonoids (TF) and hydrolysable tannins (HT).

Acid hydrolysis and extraction of phenolic aglycones

The extraction of aglycones (Ag1) was carried out according to the protocol described by Robles et *al*. [35]. Thus, 1 g of leaf samples were suspended in 80 mL 2N hydrochloric acid (HCl). The acid treatment makes it possible to generate the Agl from the O-glycosylflavones; the C-O-C bond between sugar and genin is fragile. The solution was heated at 80°C in a water bath for 50 min with air insufflation every 10 min. This method also makes it to extract certain phenolic substances, in the free state. After cooling for about 30 min, the solutions were filtered and the Agl were extracted by washing with 2 x 60 mL then 1 x 40 mL of diethyl ether. The different ether fractions are then combined and evaporated in the open air. The dry residues were taken up in 5 mL of ethanol and kept in the freezer in the dark.

Table 1. Main geographical coordinates and climatic characteristics of stations

Species	Station	Characteristics					
		Latitude	Longitude	Altitude (m)	Tm (°C)	Ann. rain (mm)	Q_2
P. lentiscus	Azeffoun (Az)	36°53' N	4°25' E	50	6.91	880	95.86
	Ouezra (O)	36°16' N	2°52' E	577	5.1	638	79.44
	Tarik Ibn Ziad (Ti)	35°94' N	2°80' E	787	6.62	524.47	54.53
P. atlantica	Thniet El Had (T)	35°52' N	1°56' E	1353	-0.07	429	41.97
	Sidi Boutouchent (S)	35°50' N	1°59' E	1240	0.64	444	43.23
	Ain Oussera (Ao)	35°52' N	2°57' E	737	3.16	349	35.96

Note: Tm (°C): Minimal temperature, Ann. Rain (mm): Annual rains: means of 10 years from 2003 to 2013 [22], Q_2 : Emberger quotient (equal 2000 P / (M^2 - m^2), where: P: Annual average rainfall (mm), M: Average of the maximum temperature of the hottest month (°K), and m: Average of the minimum temperature of the coldest month (°K) [12].

Table 2. Main chemistry characteristics of soil sampling stations

Species	Sites	Parameters		
		pH	OM (%)	$CaCO_3$ (%)
P. lentiscus	Azeffoun (Az)	7.50±0.1	2.38±0.35	6.56±0.31
	Ouezra (O)	8.76±0.1	1.5±0.09	18.12±2.1
	Tarek Ibn Ziad (Ti)	7.83±0.06	2.85±0.39	19.37±1.56
P. atlantica	Thniet El Had (T)	6.90±0.1	7.08±0.56	19.53±0.27
	Sidi Boutouchent (S)	7.08±0.1	5.5±0.33	19.50±0.97
	Ain Oussara (Ao)	8.8±0.1	1.68±0.27	18.33±0.9

OM: organic matter

Qualitative analysis of total polyphenols by HPLC-DAD

Chromatographic analysis of TPP was performed by high performance liquid chromatography (HPLC), model Agilent Technologies 1100 series (CA, USA), equipped with a quaternary pump and a G1315A automatic injector. The column is of the Hypersil™ ODS C18 type with dimensions of 5 µm, 4 x 250 mm. A binary gradient elution solvent system, of acetonitrile (A) and formic acid (B) in distilled water (98/2, v/v) was used as shown in table 3. The injected volume of the phenolic extract is 10 µL, the substances are detected using a diode array detector (DAD) which directly measures the absorbance over several wavelengths (200, 254, 280, 330 and 355 nm) at a time, these are chosen according to the maximum absorbance of the target molecules. In the case of our extract, the purity and absorbance of the peaks were detected by DAD (UV-Vis) at 280 nm; the detector placed at the outlet of the column gives a plot called a chromatogram. The levels of the molecules detected are expressed as distribution area (%), obtained by dividing the area of each peak by the sum of the areas of all the peaks in the chromatogram. The purified fractions of polyphenols were identified by matching their retention time (RT) and UV-vis absorption spectrum with those of pure substances.

Table 3. HPLC-DAD gradient solvent system for total polyphenols separation

Time (min)	Solvent A (%)	Solvent B (%)
6	35	65
9	60	40
14	80	20
25	100	0
30	35	65

Qualitative analysis of phenolic aglycones

Equipment and software

GC-MS was performed on an Agilent 7890A GC system, coupled to an Agilent 5972C mass spectroscopy detector with electron impact ionization (70 eV). A HP-5 MS capillary column (30 m x 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; Hewlett-Packard, CA, USA) was used. The temperature of the column was programmed to increase from 150°C to 220°C with a rate of 7°C/min then to 300°C with a rate of 15 °C/min and kept constant for 15 min. The carrier gas was helium N60 with a flow rate of 0.9 mL/min; split ratio was 100:1. The scan time and mass range were 1s and 50-1050 m/z, respectively. The identification of the compounds was based on mass spectra (compared with the Wiley Registry 9th Edition/NIST 2011 edition mass spectral library).

Determination of total polyphenol content

The polyphenol content in the extracts was determined using the Folin-Ciocalteu colorimetric method based on the oxidation/reduction [26]. Thus, 100 µL of standard solution (gallic acid), samples, or distilled water for blank, were transferred to test tubes and mixed. The 500 µL Folin-Ciocalteu reagent (1/10, v/v) was added, mixed, and allowed to react for 5 min before adding 150 µL of 7.5% (w/v) Na_2CO_3. The mixtures were allowed to react for 60 min at room temperature. Absorbances were measured at 740 nm using a UV-Vis Shimadzu UVmini-1240 spectrophotometer and the total phenolic content was expressed in mg Gallic acid equivalents per gram of dry weight (mg GAE g^{-1}DW) through the calibration curve with Gallic acid.

Determination of total flavonoid content

The TF content was determined according to the method described by Li et *al*. [24]. Briefly, 100 µL of each sample solution was introduced into a test tube. The volume was made up to 600 µL with distilled water and then 60 µL of $NaNO_2$ (1/20, w/v) was added. After 6 min, 60 µL of $AlCl_3$ (1/10, w/v) was added and 800 µL of 1M NaOH were added 6 min later. Then, the volume was adjusted to 2 mL with distilled water. The resulting solution was well stirred and incubated for 15 min at room temperature. Absorbances were measured at 506 nm with a UV-Vis Shimadzu UVmini-1240 spectrophotometer. TF concentrations were deduced from a standard curve prepared from rutin and calculated as mg rutin equivalent.

Determination of hydrolysable tanin content

The content of HT in the ethanolic extracts was determined by the method of Willis & Allen [46] modified by Çam & Hisil [9]. Briefly, 0.2 mL of each extract was mixed with 1.8 mL of H_2O and 1 mL of 2.5% (w/v) KIO_3. The reaction mixtures were vortexed and incubated in the dark for 30 min at room temperature. Absorbances of the red-color mixtures were read at 550 nm against a standard range of tannic acid. The content of HT was expressed in tannic acid equivalent $mg \cdot g^{-1}$ of dry matter.

Statistical analysis

All analysis were done in triplicates. Data are expressed as mean ± standard deviation (SD). Slopes and intercepts of calibration graphs were calculated by linear regression. The values are analyzed using the 1-factor ANOVA. The results are considered significantly different at $p < 0.05$. The Newman-Keurls test distinguishes groups. Principal component analysis (PCA), a multidimensional exploratory analysis, is used to determine the possible correlations between the different quantitative variables (TPP, TF, HT and Agl) and between these variables and the sample individuals. All statistical analyzis were performed using the STATISTICA 7.1 software.

RESULTS

Qualitative variability of polyphenols

The phytochemical profiles of ethanolic extracts from the leaves of *P. atlantica* Desf. and *P. lentiscus* L. obtained by HPLC-DAD at 280 nm detected the presence of fourteen compounds, of which nine were identified (table 4). Gallic acid, hydrated catechin and apigenin were identified in all samples of both species; chlorogenic acid was only detected in samples of *P. atlantica* from the Thniet El Had and Ain Oussara stations. Daidzein, an isoflavone, was only detected in *P. lentiscus* at the Azeffoun and Ouzera stations. The Aïn oussara samples with 12 compounds revealed are qualitatively the richest. These results show that *P. atlantica* is qualitatively richer than *P. lentiscus*. Indeed, 13 compounds were detected in the first and only 8 compounds in the second. *P. lentiscus*, sclerophyllous and symperverante species with leathery leaves, would orient its metabolic pathways on the one hand towards the synthesis of the constituents of the cuticle and on the other hand towards the synthesis of terpenes.

The situation is clearer by examining the GC-MS profiles of ethanolic extracts obtained after acid hydrolysis of leaf powder (fig. 1). More than twenty five compounds were detected and thirteen of them were identified; five are found only in *P. lentiscus*: ploroglucinol, β-phenylacetic acid, 4-hdroxy-phenyl-ethanol, shikimic acid.

Quantitative variability of total polyphenols, total flavonoids, hydrolysable tannins and phenolic aglycones content

TPP, TF and HT concentrations in *P. atlantica* leaf extracts are more homogeneous (table 5) and range from (26.55 ± 2.82) to (28.56 ± 3.61), from (5.71 ± 1.17) to (6.68 ± 1.14) and from (156.49 ± 9.57) to (165.09 ± 20.27) $mg.g^{-1}$ DM (dry matter) respectively compared with those of *P. lentiscus* statistically more heterogeneous ($p = 0.0000$) [(16.69 ± 2.66) to (21.71 ± 4.41), (8.38 ± 1.14) to (10.40 ± 1.4) and (67.98 ± 9.47) to (127.63 ± 15.49) $mg.g^{-1}$ DM]. Concentrations of TPP and HT were higher in the ethanolic extracts of *P. atlantica* (27.69 ± 1.03 and 160.15 ± 4.43) $mg \cdot g^{-1}$ DM respectively, than in those of *P. lentiscus* (19.30 ± 2.51 and 89.88 ± 32.83 $mg.g^{-1}$ DM, respectively) while the concentrations of TF were higher in the ethanolic extracts of *P. lentiscus* (9.50 ± 1.2 $mg.g^{-1}$ DM). The Agl concentrations show rather significant intra-specific variation with (432.72 ± 158.87) $\mu g \cdot g^{-1}$ DM in *P. atlantica* and (444.91 ± 148.58) $\mu g \cdot g^{-1}$ DM in *P. lentiscus*.

Table 4. Phenolic compounds detected by HPLC-DAD in *P. atlantica* and *P. lentiscus* ethanolic leaf extracts

Number	RT (min)	Compound	*P. lentiscus* sites	*P. atlantica* sites
1	2.65	NI	Az, O	-
2	3.31	Apigenin	Az, O, Ti	T, S, Ao
3	3.45	Gallic acid	Az, O, Ti	T, S, Ao
4	3.97	NI	-	Ao
5	4.35	Proto catechic acid	Ti	Ao
6	4.57	Hydrat catchin	Az, O, Ti	T, S, Ao
7	4.78	Tannic acid	Az, Ti	Ao
8	4.88	NI	Az, O	T, S
9	5.16	NI	-	Ao
10	5.23	Chlorogenic acid	-	T, Ao
11	5.38	NI	-	T, Ao
12	8.42	Daitzein	Az, O	Ao
13	11.94	Trans-cinnamic acid	-	Ao
14	17.73	Naringinin	-	T, Ao

Note: RT: retention time, NI: unidentified, - : absence

Table 5. Total polyphenol contents in leaf extracts of *P. atlantica* and *P. lentiscus*

Species	Sites	Content			
		TPP ($mg.g^{-1}$ DM)	TF ($mg.g^{-1}$ DM)	HT ($mg.g^{-1}$ DM)	Agl ($\mu g.g^{-1}$ DM)
P. lentiscus	Azeffoun (Az)	21.71 ± 4.41^{b}	10.40 ± 1.40^{a}	127.63 ± 15.49^{b}	615.28 ± 31.82^{a}
	Ouzra (O)	16.69 ± 2.66^{c}	08.38 ± 1.14^{b}	67.98 ± 9.47^{c}	342.36 ± 51.05^{bc}
	Tarik Ibn Ziad (Ti)	19.50 ± 2.51^{ab}	09.73 ± 0.91^{ab}	74.04 ± 7.77^{c}	377.08 ± 45.54^{bc}
P. atlantica	Thniet El Had (T)	26.55 ± 2.82^{a}	05.71 ± 1.17^{c}	158.88 ± 10.56^{a}	441.90 ± 56.97^{b}
	Sidi Boutouchent (S)	27.98 ± 2.62^{a}	06.68 ± 1.14^{c}	165.09 ± 20.27^{a}	586.81 ± 24.34^{a}
	Ain Oussara (Ao)	28.56 ± 3.61^{a}	05.81 ± 1.73^{c}	156.49 ± 9.57^{a}	269.44 ± 84.74^{c}

Note: Values are given as mean ± SD. Means marked with the same letter do not differ significantly ($P > 0.05$).

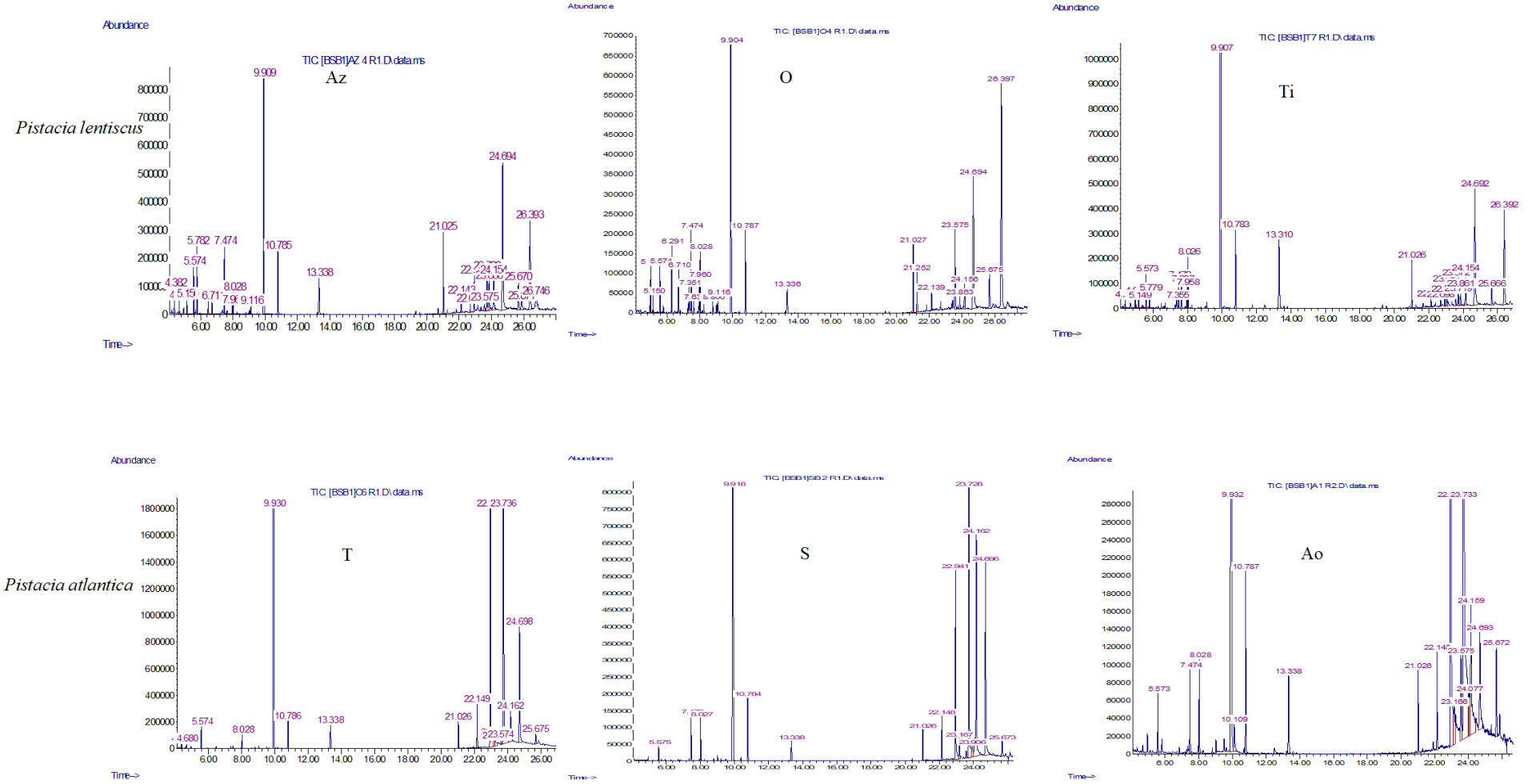

Fig. 1. GC-MS profils of *P. atlantica* and *P. lentiscus* ethanolic leaf extracts in study sites : Thniet El Had (T), Sidi Boutouchent (S), Ain Oussara (Ao), Azeffoun (Az), Ouzera (O), Tarik-Ibn-Ziad (Ti)

DISCUSSION

To cope with the arid conditions of summer, Mediterranean species like *P. lentiscus*, can adopt a tolerance strategy allowing them to perform their normal functions, even under conditions of water shortage [8]. According to Ait Said [2] *P. lentiscus* totaled 49 terpene compounds and only 41 in *P. atlantica*. The latter being deciduous and with a finer cuticle [5], it promotes the synthesis of phenolic compounds rather than other constituents. Species of the genus *Pistacia*, xerophytic species, present various forms of adaptation [2, 5] reflecting good phenotypic plasticity. Gallic acid and myricetin derivatives have already been identified in *P. lentiscus* [36, 38], while daidzein, chlorogenic acid and apigenin have not been reported. The luteolin, quercetin and kaempferol derivatives were not revealed in our samples by HPLC either because they are absent or present at undetectable concentrations in the samples or because they do not absorb at 280 nm [19]. Romani et *al*. [38] reported the absence of kaempferol derivatives and found a distinct *P. lentiscus* in the family Anacardiaceae. Vaya & Mahmood [45] and Rodríguez-Pérez et *al*. [36] detected them in samples of *P. lentiscus* from Israel and Bejaia respectively, which suggesting the presence of several ecotypes. According to Cheynier [10], the diversity of structures is associated with their specific roles in plants, hence their specific distribution. Thus, shikimic acid for example, would delay leaf senescence by stimulating the biosynthesis of chlorophylls [4]. Its accumulation in the leaves of *P. lentiscus* indicates that it is not only an intermediate of the shikimate pathway but is likely to be a physiological end product. The (-)catechin excreted in root exudates acts as an allelopathic substance inhibiting the germination of native species [6]. In addition, phloroglucinol, a degradation product of phloredzine, is known for its growth regulator properties [41, 43, 44] and to promote the proliferation of axillary shoots [43]. This compound is absent in all samples of *P. atlantica*, an arboreal species. In order to understand the variability within and between the two species and its relation to certain environmental factors, PCA analysis was performed. The first component (PC_1) accounted for 54.4% of the total variance of the data set and PC_2 accounted for 23.07%, with a combined total of 77.5%. Grouped variables were strongly positively correlated (TPP, HT, "Gal.Ac +Api").The variable FT is negatively correlated with almost phenolic variables (fig. 2). The Agl variable contributes mainly to the formation of PC_2 and is virtually unrelated to any other variables.

This analysis shows that climatic and edaphic parameters projected in supplementary variables were relatively correlated with the phenolic variables: TPP, HT and Gal.Ac+Api were positively correlated to altitude (Alt.), soil organic matter (S.OM), soil $CaCO_3$ ($S.CaCO_3$) and negatively correlated to Q_2 whereas TF performed inversely with these parameters.

Overall, populations of *P. atlantica* projected on the negative side of PC_1, with high levels of TPP and HT and lower level of TF, are opposite to those of *P. lentiscus* projected on the positive side of PC_1 with lower levels of TPP and HT and high level of TF.

The projection of individuals (fig. 3) according to the PC_2 show relative heterogeneity reflecting intra-specific variability of Agl. This confirms the aglycone heterogeneity of the species found by analysis of variance (p= 0.0000).

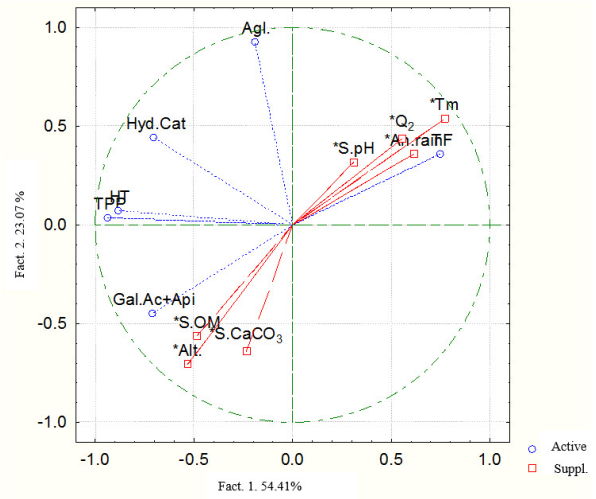

Fig. 2. Projection of the active variables and illustrative variables in the principal plane (first and second axes planes)

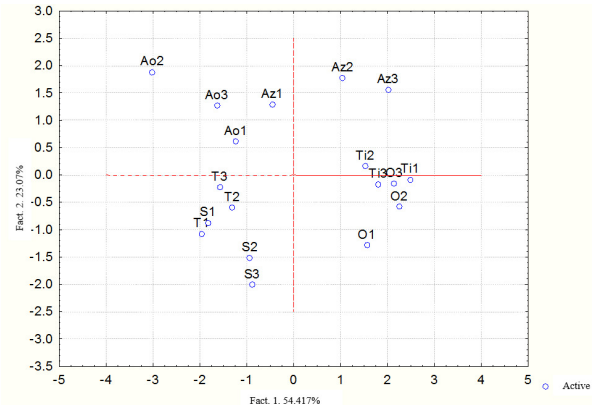

Fig. 3. Projection of the individuals of *P. lentiscus* and *P. atlantica* in the principal plane: Azeffoun (Az), Ouzera (O), Tarik-Ibn-Ziad (Ti), Thniet El Had (T), Sidi Boutouchent (S), Ain Oussara (Ao) and Ouzera (O) individuals

The polyphenolic variations could be of genetic origin [30] or environmental [40]. According to Gobbo-Neto and Lopes [18], the response of plants to different environmental factors is related to the role of each class of phenolic compounds. Thus, flavonoids synthesis increases with the intensity of UV radiation while the tannins and the simple phenolic acids which absorb at shorter wavelengths, remain unchanged. This suggests that climatic and edaphic factors could influence the synthesis of phenolic compound. Indeed, soil exerts a strong selective pressure and adaptation of plant species and consequently on the distribution of

vegetation [33]. This suggestion is corroborated by the results of Kraus et *al*. [20] that the levels of polyphenols and condensed tannins in the leaves increase strongly as a result of soil nutrient deficiency. The Giel and Bojarczuk [17] experiments also showed an increase in leaf polyphenol concentrations in *Rhododendron* with an increase in $CaCO_3$ content while Li et *al*. [23] found an increase in TF in poplar leaves with a drop in temperature.

In conclusion, the phytochemical profiles of all populations of *Pistacia* genus detected fourteen phenolic compounds by HPLC-DAD and more than twenty five by GC-MS; these results show phenolic heterogeneity in *P. lentiscus* populations and relative homogeneity in those of *P. atlantica*. In addition, the analysis of the relationships between the chemical compounds of the populations examined and the climatic and edaphic factors of their respective habitats showed the influence of the latter on the biosynthesis of polyphenols. The contents of total polyphenols (TPP) and hydrolyzable tannins (HT) increase with altitude, soil organic matter and soil $CaCO_3$; while those of total flavonoids (TF) increase with precipitation. In perspective, an exhaustive identification of the polyphenols of the two species is desirable in order to better understand the strategies of adaptation to environmental conditions, in particular the individual variations according to the constraint, which could enrich the work in progress.

Acknowledgements. The authors would like to warmly thank Professor Arezki Derridj teacher and researcher at Mouloud Mammeri University in Tizi-Ouzou, Algeria for his scientific collaboration. They also wish to thank Ms Sawssen Selmi and Majdi Hammami engineer for bioactive substances laboratory of Borj Cédria (Tunisia) for their technical contribution.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Agati, G., Brunetti, C., Ferdinando, M.D., Ferrini, F., Pollastri, S., Tattini, M., (2013): Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. Plant Physiology and Biochemistry, 72: 35-45.

[2] Ait Said, S., Fernandez, C., Greff, S., Torre, F., Derridj, A., Gauquelin, T., Mevy, J.P., (2011): Inter-population variability of terpenoid composition in leaves of *Pistacia lentiscus* L. from Algeria: a chemoecological approach. Molecules, 16: 2646-2657.

[3] Allam, A., Moussa, R., Najem, W., Bocquillon, C., (2019): Mediterranean specific climate classification and future evolution under RCP scenarios. Hydrology and Earth System Sciences Discussions, https://doi.org/10.5194/hess-2019-381.

[4] Aldesuquy, H.S., Ibrahim, A.H.A., (2000): The role of shikimic acid in regulation of growth, transpiration, pigmentation, photosynthetic activity and productivity of *Vigna sinensis* plants. Phyton (Horn, Austria), 40(2): 277-292.

[5] Al-Saghir, M.G., Porter, D.M., Nilsen, E.T., (2006): Leaf anatomy of *Pistacia* species (Anacardiaceae). Journal of Biological Sciences, 6(2): 242-244.

[6] Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M., Vivanco, J.M., (2003): Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science, 30(5): 1377-1380.

[7] Ben Haj Yahia, I., Zaouali, Y., Ciavatta, M.L., Ligresti, A., Jaouadi, R., Boussaid, M., Cutignano, A., (2019): Polyphenolic profiling, quantitative assessment and biological activities of tunisian native *Mentha rotundifolia* (L.) Huds. Molecules, 24(2351): 1-19.

[8] Bussotti, F., Piccini, C., Piotto, B., Cervelli, C., (2005): La macchia Mediterranea: la vegetazione e il suo rapporto con l'uomo. *In:* Le specie arbustive della macchia Mediterranea un patrimonio da valorizzare. Collana Sicilia Foreste, 26: 11-36.

[9] Çam, M., Hisil, Y., (2010): Pressurised water extraction of polyphenols from pomegranate peels. Food Chemistry, 123: 878-885.

[10] Cheynier, V., (2012): Phenolic compound: from plants to foods. Phytochemistry Reviews, 11: 153-177.

[11] Correia, O., Diaz Barradas, M.C., (2000): Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. Plant Ecology, 149: 131-142.

[12] Daget, P., (1977): Le bioclimat méditerréen: analyse des formes climatiques par par le système d'Emberger. Vegetatio 34(2): 87-103.

[13] Dai, J., Mumper R. J., (2010): Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules, 15: 7313-7352.

[14] FAO and Plan Bleu (2018): State of Mediterranean Forests 2018. Food and Agriculture Organization of the United Nations, Rome and Plan Bleu, Marseille, 308 p.

[15] Flexas, J., Diaz-Espejo, A., Gago, J., Galle, A., Galmes, J., Gulias, J., Medrano, H., (2014): Photosynthetic limitations in Mediterranean plants: A review. Environmental and Experimental Botany, 103: 12-23.

[16] Freschet, G.T., Violle, C., Roumet, C. & Garnier, E. (2018) Interactions entre le sol et la végétation : structure des communautés de plantes et fonctionnement du sol. Les sols au cœur de la zone critique : écologie (eds P. Lemanceau & M. Blouin), pp. 83-99. ISTE editions, London, UK.

[17] Giel, P., Bojarczuk, K., (2011): Effects of high concentrations of calcium salts in the substrate and its ph on the growth of selected Rhododendron cultivars. Acta Societatis Botanicorum Poloniae, 80(2): 105-114.

[18] Gobbo-Neto, L., Lopes N.P., (2007): Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quimica Nova, 30(2): 374-381.

[19] Harborne, J.B., (1998): Phenolic compound *in*: Phytochemical methods a guide to modern techniques of plant analysis. 3rd Ed. Chapman & Hall., pp. 40-96.

[20] Kraus, T.E.C., Zasoski, R.J., Dahlgren, R.A., (2004): Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. Plant and soil journal, 262: 95-109.

[21] Lefebvre, T., Millery-Vigues, A., Gallet, C., (2016): Does leaf optical absorbance reflect the polyphenol content of alpine plants along an elevational gradient? Alpine Botany, 126(2): 177-185.

[22] Le temps, 46 187 prévisions de temps pour toutes les villes, tous les villages et toponymes [en ligne] disponible sur « http://dz.freemeteo.com » consulté en novembre 2015.

[23] Li, C.M., Wang, Y., Yu, W.X., (2011): Dynamic changes of phenolic compound contents in leaf and bark

of poplar during autumn temperature drop. Journal of Forestry Research, 22(3): 481-485.

[24] Li, S., Zhu, R., Zhong, M., Zhang, Y., Huang, K., Zhi, X., Fu, S., (2010): Effects of ultrasonic-assistant extraction parameters on total flavones yield of *Selaginella doederleinii* and its antioxidant activity. Journal of Medicinal Plants Research, 4(17): 1743-1750.

[25] Mahmoudi, S., Khali, M., Mahmoudi, N., (2013): Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). Nature & Technologie, 9: 35-40.

[26] Medina, M.B., (2011): Simple and rapid method for the analysis of phenolic compounds in beverages and grains. Journal of Agricultural and Food Chemistry, 59: 1565-1571.

[27] Mocan, A., Crișan G., Vlase, L., Crișan, O., Vodnar, D.C., Raita, O., Gheldiu, A.M., Toiu, A., Oprean, R., Tilea, I., (2014): Comparative studies on polyphenolic composition, antioxidant and antimicrobial activities of *Schisandra chinensis* leaves and fruits. Molecules, 19: 15162-15179.

[28] Munné-Bosch, S., Alegre, L., (2000): Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus offcinalis* plants. Planta, 210: 925-931.

[29] Pandey, G., Khatoon, S., Pandey, M.M., Rawat, A.K.S., (2018): Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities. Journal of Ayurveda and Integrative Medicine, 9: 169-176.

[30] Pilepic, K.H., Males, E., (2013): Quantitative analysis of polyphenols in eighteen *Hypericum* taxa. Periodicum Biologorum, 115(3): 459-462.

[31] Quezel, P., Santa, S., (1963): Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II, édition Centre National de la Recherche Scientifique, Paris, France. 1170 p.

[32] Quezel, P., Medail, F., (2003): Ecologie et biogéographie des forêts du bassin méditerranéen. Ed. Elsevier, collection environnement, 573 p.

[33] Rajakaruna, N., Boyd, R.S., (2008): The edaphic factor. In Jorgensen, S.E., Fath, B. (Eds.): The Encyclopedia of Ecology, 2: 1201-1207.

[34] Ramakrishna, A., Ravishankar, G.A., (2011): Influence of abtiotic stress signals on secondary metabolites in plants Plant Signaling & Behavior, 6(11): 1720-1731.

[35] Robles, C., Greff, S, Garzino, S., Bousquet-Mélou, A., Fernandez, C., Korboulewsky, N., Bonin, G., (2003): Phenols and flavonoids in aleppo pine needles as bioindicators of air pollution. Journal of Environmental Quality, 32: 2265-2271.

[36] Rodríguez-Pérez, C., Quirantes-Piné, R., Amessis-Ouchemoukh, N., Madani, K., Segura-Carretero, A., Fernández-Gutierrez, A., (2013): A metabolite-profiling approach allows the identification of new compounds from *Pistacia lentiscus* leaves. Journal of Pharmaceutical and Biomedical Analysis, 77: 167-174.

[37] Rohling, E.J., Grant, K.M., Roberts, A.P., Larrasoan, J.C., (2013): Paleoclimate variability in the mediterranean and red sea regions during the last 500,000 years implications for hominin migrations. Current Anthropology, 54(8): 1-19.

[38] Romani, A., Pinelli, P., Galardi, C., Mulinacci, N., Tattini, M., (2002): Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L.. Phytochemical Analysis, 13: 79-86.

[39] Sahnoune, F., Belhamel, M., Zelmat, M., Kerbachi, R., (2013): Climate change in Algeria: vulnerability and strategy of mitigation and adaptation. Energy Procedia, 36: 1286-1294.

[40] Siracusa, L., Patanè, C., Avola, G., Ruberto, G., (2012): Polyphenols as chemotaxonomic markers in italian "long-storage" tomato genotypes. Journal of Agricultural and Food Chemistry, 60: 309-314.

[41] Sarkar, D., Naik, P.S., (2000): Phloroglucinol enhances growth and rate of axillary shoot proliferation in potato shoot tip cultures *in vitro*. Plant Cell, Tissue and Organ Culture, 60: 139-149.

[42] Stamatopoulos, K., Chatzilazarou, A., Katsoyanno, E., (2014): Optimization of multistage extraction of olive leaves for recovery of phenolic compounds at moderated temperatures and short extraction times. Foods, 3: 66-81.

[43] Steephen, M., Nagarajan, S., Ganesh, D., (2010): Phloroglucinol and silver nitrate enhances axillary shoot proliferation in nodal explants of *Vitex negundo* L. – an aromatic medicinal plant. Iranian Journal of Biotechnology, 8(2): 82-89.

[44] Tan, S.N., Tee, C.S., Wong, H.L., (2018): Multiple shoot bud induction and plant regeneration studies of *Pongamia pinnata*. Plant Biotechnology, 35: 325-334.

[45] Vaya, J., Mahmood, S., (2006): Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). BioFactors, 28: 169-175.

[46] Willis, R.B., Allen, P.R., (1998): Improved method for measuring hydrolyzable tannins using potassium iodate. Analyst, March, 123: 435-439.

[47] Yi, T., Wen, J., Golan-Goldhirsh, A., Parfitt, D.E., (2008): Phylogenetics and reticulate evolution in *Pistacia* (Anacardiaceae). American Journal of Botany, 9(2): 241-251.

Received: November 27, 2020
Accepted: May 27, 2021
Published Online: June 4, 2021
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House