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CONTRIBUTIONS TO THE PHYTOCHEMICAL STUDY OF SOME SAMPLES OF *AJUGA REPTANS* L. AND *AJUGA GENEVENSIS* L.

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Abstract: *Continuing a series of investigations regarding the chemical variability of some vegetal species, we initiated a comparative chemical study of the Ajuga reptans L. and Ajuga genevensis L. species used in Romanian folk medicin. We investigated the iridoidic, flavonoidic fractions as well as those of the polyphenolcarboxylic acids with the help of such investigation techniques as: thin layer chromatography, spectrophotometry and high performance liquid chromatography. The analysed vegetal material was made up of the aerial parts of the two Lamiaceae species, prelevated from individuals belonging to some natural populations from the North of Moldavia. We could notice that Ajuga reptans is richer in antimflamatory, immunomodulating and hepatoprotecting iridoids than Ajuga genevensis, while the second has a slightly higher content of antioxidant and diuretic polyphenols. In the same time, we could notice the existence of an interspecific variability due to some genetic causes, but also intraspecific, of a pedo-climatic nature. By means of HPLC we proved the presence in the studied vegetal material of chlorogenic and caffeic acid, of apigenol and luteolin-7-O-glucosyde.*

Key words: *Ajuga reptans L., Ajuga genevensis L., chemical variability, iridoids, polyphenols.*

Introduction

Ajuga reptans L. is aplant used in the traditional medicine of many countries from the centre and, especially, the eastern part of Europe. The extracts obtained from bugle (*Ajuga reptans* L.) are used due to the content of polyphenols of the flavonoidic and polyphenolcarboxylic acids type (due to its antioxidant, vascular and antimicrobial qualities), as well as of iridoids (antiinflamatory and wound healing) as antidiarrhoeaic, antileucoreic, hepatoprotecting and vulnerar. Unlike bugle, blue bugleweed, *Ajuga genevensis* L., is used only in our country as a substitute of the medicinal species, as, due to the fact that it is not very demanding as

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to the pedoclimatic conditions, it is largely outspread in the wild flora.

In our researches, we have comparatively analysed the chemical composition of four samples *Ajuga reptans* L. And five of *Ajuga genevensis* L. harvested at the beginning of May 2010 in the north eastern part of Moldavia.

The objective of the investigation was to establish the chemical similarities and differences between the two species especially regarding the composition of the irridoidic, flavonoidic and polyphenolic acid fractions.

The aims of the phytochemical study were to determine the biosynthetic level of the flavonoidic, iridoidic or caffeic/chlorogenic acid type derivates, as well as the influence of some genetic factors (the belonging of the species to the same genus) or environmental (soil and climate), particular and different, also depending on the location of the analysed natural population. Our studies were performed on the vegetal material harvested in 2010.

2. Material and Method

The vegetal material of *Ajuga reptans* L. and *Ajuga genevensis* L. samples harvested at the beginning of May 2010 from natural populations identified in the north of Moldavia was dried at room temperature and extracted with absolute methanol (DER=0.5:100g/mL), at warm temperature, till exhaustion. These extracts served to achieve the spectrophotometric determinations of polyphenols and iridoids.

The TLC and HPLC analyses were performed on extracts for which the ratio drug/extract (DER) was of 3:100 g/mL. The analysed samples were prelevated due to the data in table 1.

Table 1

The origin of the vegetal material (2010)

Sample code	Location	Altitude (m)	Collection date
Ajuga reptans L 2010			
AR1	Guranda	171	04.05
AR2	Baisa	282	04.05
AR3	Bicaz-Baraj	560	06.05
AR4	Potoci	648	06.05
Ajuga genevensis L 2010			
AG1	Albesti	70	04.05
AG2	Draslea	100	04.05
AG3	Stanceni	176	04.05
AG4	Baisa	282	04.05
AG5	Grozavesti	530	04.05

To get an idea of the iridoidic and triterpenic spectrum existing in the prelevated plants, we initially achieved a TLC study using the exhaustings with DER 3:100 g/mL [2].

The spectrophotometric determinations aimed the biosynthetic level of the above mentioned secondary metabolites, the analysed methanolic extracts having the DER 0.5:100 g/mL. In these extracts we determined the flavonoids by a treatment with aluminium chloride when the bioactive components form internal complexes with Al^{3+}, intensly yellow coloured, for which the extinction was read on the spectrophotometer at λ=413nm, using luteolin as standard [3]. The polyphenolic acid dosing was performed by treating the extracts with phosphowolframic acid in an alkaline medium, when we obtained blue colourings, colourimetered at λ=660nm, compared to the chlorogenic standard [3]. **The iridoids** extracted from the vegetal product and purified by passing the extract over a silicagel column, were treated with PABA in acid medium, when, at warmth, there forms a blue coloured compound to be colourimetered at λ=590 nm, the standard curve being traced with aucuboside [4, 5, 6, 7]. The methanolic exhaustions for which DER was equal to 3:100g/mL were also qualitatively

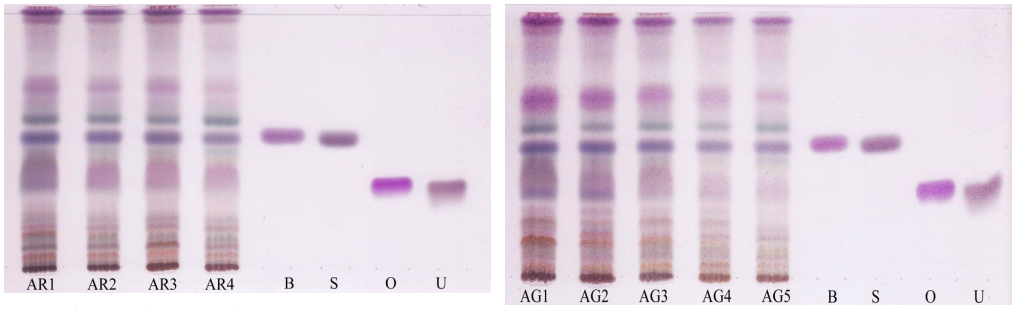
and half-quantitatively analysed by HPLC for polyphenols.

Chromatography conditions: an HPLC Agilent 1100 system with registration and integration of the chromatograms by means of the Borwin computerized system and multidiode detector.

The stationary phase: eclipse XDB-C18 (150mm x 4,6mm; 5µm); The mobile phase: component A=sodium acetate 2mM brought at the pH=2.5 with acetic acid; component B=acetonitryl; gradient: 98% A;2% B (0-20 min.); 86% A:14%B (20-40 min.); 80%A:20% B (40-50 min); 70%A:30B (50-60 min.); 98%A:2%B (after 65 min.); flow: 1mL/min.; temperature in the column compartment 25^0C; detection: UV 320nm for samples and standards; in addition for each standard we registered the absorbtion spectrum in the interval of 200-400nm; applied volume: 100µL fir the solutions to be analysed and the standard.

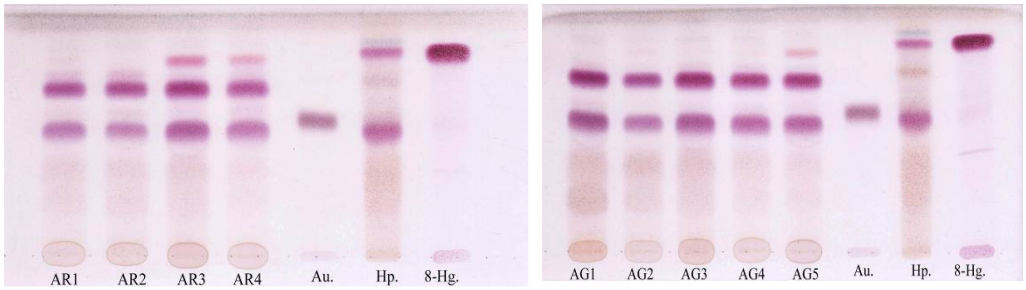
Results and Discussions

The important part of our study was the comparative phytochemical analysis of the two *Ajuga* species to draw conclusions regarding the inter and intraspecific chemical variability. To monitorize the similarities and differences of chemism in the triterpenic and iridoidic fractions, we analysed the two methanolic *Ajuga* exhausts by the TLC method. In fig.1 and 2 are the thin layer chromatograms for the terpenes and iridoids of the *Ajuga reptans* and *Ajuga genevensis* samples.



Legend: samples- v. tab.1;
standards: β-sitosterol (B), stigmasterol (S), oleanolic acid (O), ursolic acid (U)

Fig. 1. *TLC for terpenes of „Ajuga reptans" and „Ajuga genevensis" samples*



Legend: samples-v. tab.1;
standards: aucuboside (Au), harpagide (Hp), 8-O-acetyl harpagide (8-Hg)

Fig. 2. *TLC for iridoids of „Ajuga reptans" and „Ajuga genevensis" samples*

Regarding the iridoids identified in the *Ajuga* extracts, they seem to be represented by two components in both species, out od which the inferior one (Rf=0.53) seems to be harpagide, and the second remaining unknown due to the lack of the corresponding stamdard. A third spot, superior but slight, appears in the case vof two *Ajuga reptans* samples (AR3 and AR4) and one *Ajuga genevensis* (AG5) and seems to belong to the 8-O-acetyl harpagide.

If from TLC study produced the main conclusion was the confirmation of the similar chemism in the same species, the highlighting of the differences between the two species respectively, the spectrophotometric dosings of the flavonoids, polyphenolic acids and iridoids produced differenciated quantitative assertions. In tablel 2 we present the results obtained by the quantitative determinations.

Table 2

The content in iridoids and polyphenols dosed in vegetal material (Ajuga reptans L./Ajuga genevensis L.) prelevated from different natural populations in 2010

Sample Code	**Origin**	**Determination in 100g herba**		
		Iridoids	**Flavonoids**	**Polyphenolic acids**
		g aucubozid %	**g luteolin %**	**g chlorogenic acid %**
***Ajuga reptans* L.**				
AR1	Guranda	1.329 ± 0.0097	0.501 ± 0.0034	1.581 ± 0.0022
AR2	Baisa	1.078 ± 0.0032	0.471 ± 0.0047	1.690 ± 0.0016
AR3	Bicaz – Baraj	1.983 ± 0.0010	0.455 ± 0.0066	1.870 ± 0.0013
AR4	Potoci	1.673 ± 0.0085	0.563 ± 0. 0019	1.744 ± 0.0031
***Ajuga genevensis* L.**				
AG1	Albesti	0.988 ± 0.0059	0.698 ± 0.0012	2.020 ± 0.0074
AG2	Draslea	0.8763 ± 0.0036	0.839 ± 0.0011	1.876 ± 0.0061
AG3	Stauceni	0.641 ± 0.0071	0.561 ± 0.0093	1.825 ± 0.0002
AG4	Baisa	1.033 ± 0.0081	0.417 ± 0.0055	1.593 ± 0.0035
AG5	Grozavesti	0.9512 ± 0.0099	0.545 ± 0.0043	1.733 ± 0.0006

As may be noticed, the *Ajuga reptans* samples are richer in immunomodulating and intiimflamatory iridoids, while in case of *Ajuga genevensis* the antioxidant polyphenol content is higher than in the case of bugle.

If we graphically represent the values from the table, we will notice that regarding the iridoids (fig. 3) the richest sample proved to be *Ajuga reptans* hervested at Bicaz-Baraj, the other bugle samples having a lower content.

Compared to this intraspecific variability determined for the iridoidic fraction of bugle, *Ajuga genevensis* presents a similar situation, yet this time the highest non-volatile monoterpen content being found in the Baisa sample, and the most reduced in that of Stauceni.

On the other hand, it is obvious that along with the intraspecific variability, there also is a genetic, interspecific, variability, *Ajuga reptans* being much richer in iridoids than blue bugleweed. Regarding the flavonoids, (fig. 4), we notice that the richest sample in such compounds is that of *Ajuga genevensis* from Draslea, *Ajuga reptans* containing smaller flavonoid quantities.

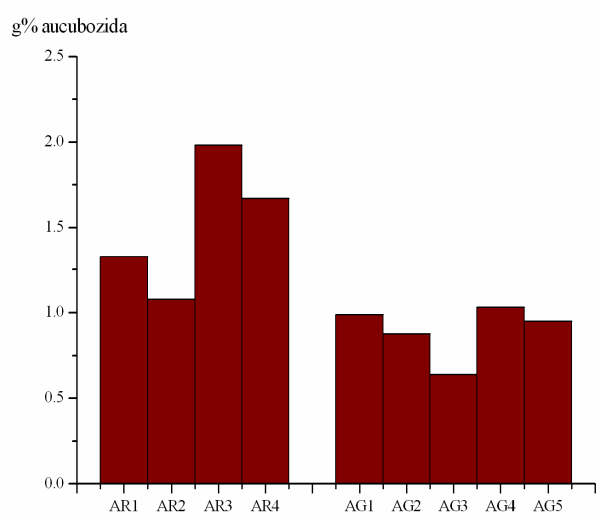


Fig. 3. *The graphic expression of the iridoid content determined in the „Ajuga" exhausts* (2010)

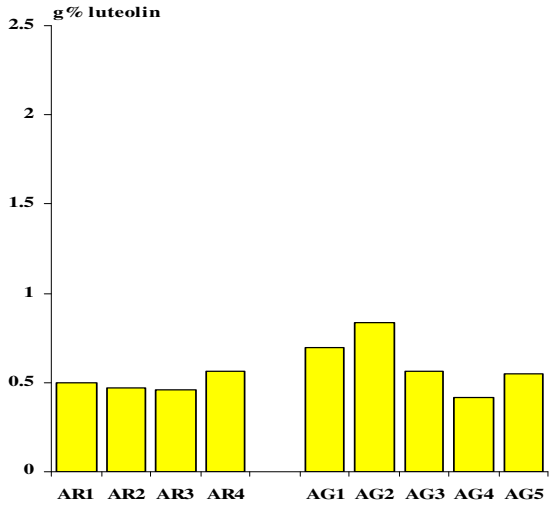


Fig. 4. *The graphic expression of the flavonoid content determined in the "Ajuga" exhausts* (2010)

Comparing the values of the Baisa *Ajuga reptans* with those of *Ajuga genevensis* of the same location, we notice that the quantitative differences in case of the three fractions of active principles are not major (fig. 5), for the same location, the climatic and soil conditions beinf identical for the two species.

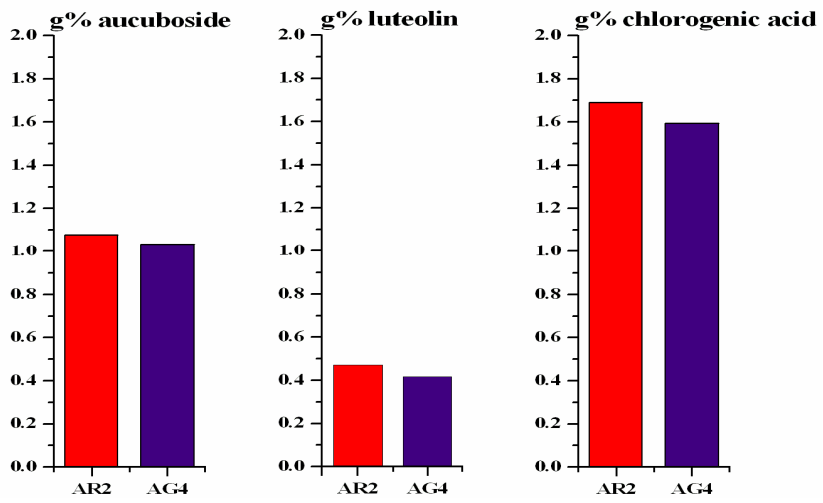


Fig. 5. *The graphic expression of the iridoid and polyphenol content determined in methanolic extracts (DER 0,5:100) of bugle and blue bugleweed harvested in the same location-Baisa-in 2010*

To better appreciate the similaritiesa and differences of the polyphenolic spectrum of the nine *Ajuga* samples, we resorted to the HPLC analysis. In table 3 we present the components as well as the corresponding quantities identified the polyphenols in exhausts (DER 3:100 g/mL).

Table 3

Polyphenols identified in Ajuga reptans and Ajuga genevensis methanolic exytracts (2010)

Sample code	**Chlorogenic acid**		**Caffeic acid**		**Luteolin 7-O-glucoside**		**Apigenol**	
	ug/mL	mg/100g	ug/mL	mg/100g	ug/mL	mg/100g	ug/mL	mg/100g
Ajuga reptans L								
AR1	3.62	12.08	0.97	3.25	0.35	1.17	1.43	4.76
AR2	1.52	5.07	0.97	3.25	0.14	0.48	0.63	2.11
AR3	2.97	9.90	1.23	4.10	0.09	0.31	3.07	10.25
AR4	2.03	6.77	1.32	4.39	0.15	0.84	1.30	4.33
Ajuga genevensis L								
AG1	4.21	14.05	1.06	3.55	0.51	1.72	1.49	4.98
AG2	9.73	32.44	1.60	5.34	0.94	3.15	3.90	12.99
AG3	6.01	20.05	1.73	5.77	1.00	3.32	2.83	9.43
AG4	44.68	148.93	0.83	2.77	1.55	5.16	3.15	10.50
AG5	7.08	23.61	2.07	6.90	1.53	5.10	3.94	13.15

Calculating the concentrations of the chlorogenic acids, caffeic acids, luteolin-7-O-glucoside and apigenol existing in the 100 g of vegetal product, we obtained the values indicated by the same table 3. Expressing these concentrations graphically, we have the situation from fig. 6.

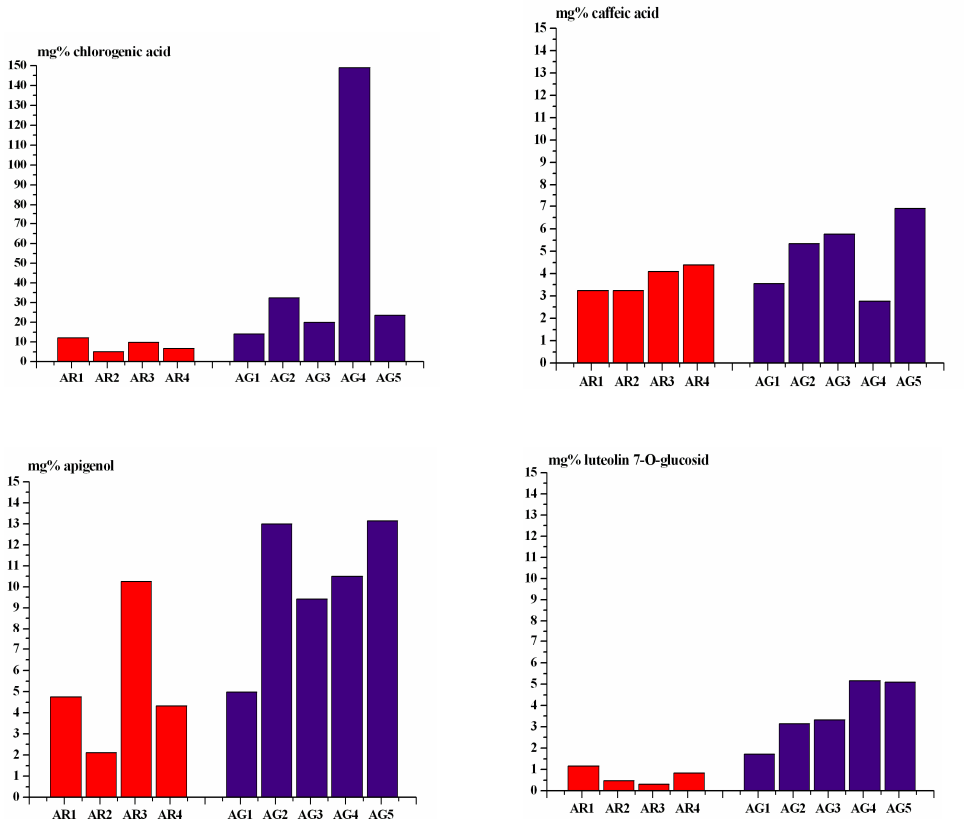


Fig. 6. *The variation of the content in chlorogenic acid, caffeic acid, luteolin-7-O-glucoside and apigenol (g/100g herba) determined by HPLC in the samples of „Ajuga reptans" and „Ajuga genevensis"* (2010)

One can notice that of the two poliphenolic acids, the chlorogenic one is quantitatively better represented, there appearing, as already seen, great intraspecific variations, yet espacially interspecific. The second poliphenolic acid, the caffeic acid has a smaller concentration, still the *Ajuga genevensis* are richer in this component. Coming back to the achieved spectrophotometric determinations, we will notice that the majority of the components of the group were not identified. Thus, we have the question of how many more, not identified acids, are there in the vegetal products.

Analogous, we notice that out of the flavonoidic fraction, we could identify only two components: apigenol and luteolin-7-O-glucoside. In this case, apigenol dominates, yet we again notice that the *Ajuga genevensis*

populations seem to be richer in this flavonic aglicon than those of *Ajuga reptans.* Again, there arises the question of what flavonoids coexist in *Ajuga genevensis* L. and *Ajuga reptans* L.

Conclusions

The comparative phytochemical study on the two *Ajuga* species, *Ajuga reptans* and *Ajuga genevensis* aimed to highlight the qualitative and quantitative variations of the iridoidic, flavonoidic and polyphnolic acids fractions in dry vegetal material. We noticed that, in both species, the individuals originated from populations developed in different locations, present variations of larger or more restricted limits especially quantitative and less qualitative for the same group of secondary metabolites.

Ajuga reptans proved to be richer in iridoidic components of the harpagide type (antiinflamatory, hepatoprotecting and immunomodulating) while *Ajuga genevensis* is slightly richer in polyphenols (antioxidant and diuretic). Out of the polyphenolic components, by HPLC, we identified and quantified chlorogenic acid, caffeic acid, apigenol and luteolin-7-O-glucoside.

Aknowledgement

The paper was presented at the: al IV lea Simpozion National de Etnofarmacologie cu participare internationala cu tema "Etnofarmacologia la interfata bioaliment-fitomedicament" - iunie 2011 Brasov – Sirnea.

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