UNIVERSITY "ALEXANDRU IOAN CUZA" IASI
FACULTY OF BIOLOGY

RESEARCHES REGARDING THE VARIABILITY OF THE METABOLOM, TAXONOMICALLY DETERMINED, OF MEDICINAL AND AROMATIC PLANTS

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Abstract/Summary

Key words: medicinal plants, metabolom, taxonomically, histo-anatomical, inter- and intraspecific variability.

The reason of choosing the doctoral thesis with the above mentioned title resides in the fact that the medicinal and aromatic plants were, up to the beginning of the XXth century, the primary source to obtain medicine, with the help of which all the diseases were treated.

Today, a great number of plants known as being medicinal entered the so called **cultured medicine**, being frequently used as raw material in the pharmaceutical industry as well as to obtain food supplements, cosmetics and hygiene preparations.

Along with the setting up of the European Community, there appeared a specific legislation referring to medicine, a separate group being made up of medicine of vegetal origin. Analogically, a common legislation for food supplements, with a vegetal component, was elaborated. As Romania joined the European Community in 2007, the phytomedicines and the food supplements must suit the general norms valuable for united Europe.

Reading the European norms on this theme, one noticed that a special accent is on the quality of these preparations, that derive from obeying the origin of the raw material, the correct identification of the vegetal species, of the organ that is to be processed, of the most propitious harvesting moment, all the mentioned requirements contributing to the outlining of a

certain chemical composition that may assure or not the desired pharmacological effect.

Regarding the chemical composition of a species, (harvested at a certain moment in the vegetation period), it is easily to be controlled in case of cultivated plants compared to those harvested from the wild flora.

In the last decade, the chemical composition determined for a certain plant in the moment of prelevation is known under the name of **metabolom**, namely the totality of the identifiable components resulting in the metabolism. It is true that, in the past two years, there have been authors to plead in favour of giving up the term metabolom.

Taking this context into account, we aim in this thesis to study a series of plants (that are used in the Romanian and European folk medicine or in the form of food supplements) from the wild flora in the North-Eastern part of Moldavia from the point of view of identifying the area of existing, of the morphological and biochemical characters and of the possibilities of protecting them by transfer into culture.

In this respect, **the aim** of our investigations was made of:

- taxoniomic and morpho-anatomical investigation and characterization of some natural populations of:
  - *Ajuga reptans* L. / *Ajuga genevensis* L.,
  - *Galium verum* L. */ Galium album* Mill.,
  - *Betonica officinalis* L.,
  - *Verbascum phlomoides* L. / *Verbascum thapsiforme* Schrad.;
- performing the phytochemical study of the iridoidic, polyphenolic and terpenoidic fractions
- identifying the existence of a morohological and biochemical inter- and intraspecific variability;
- identifying some basins in North-Eastern Moldavia in which the studied plants are in abundance;

- ❑ the transfer of some *Ajuga reptans* individuals from the wild flora into culture.

The objectives of achieving these aims were:

- ❑ establishing the morpho-anatomical characters for the species included in the study;
- ❑ achieveing the biometry to characterize the spontaneous populations belonging to the *Ajuga, Betonica, Verbascum* species*;*
- ❑ establishing the chemical qualitative and quantitative profile of the iridoidic and polyphenolic fractions and the volatile oil for *Ajuga;*
- ❑ bringing into culture some individuals of *Ajuga reptans* L.

The work presented to obtain the title of Phd in biological sciences is made up of two parts:

- a **general part** that comprises one chapter and
- a **personal part** having six chapters, so that the work is structured on the following

CONTENT

2.1.2. The vegetal material of *Galium verum* L. and *Galium album* Mill.

2.1.3. Presentation of the vegetal material of the *Betonica officinalis* L. species

2.1.4. The vegetal material studied in the case of the *Verbascum phlomoides* L. and *Verbascum thapsiforme* Schrad. species

2.2. Histo-anatomical researches

2.2.1. Methods and techniques used in the histo-anatomical researches on the anatomical apparatus of the plants harvested from the wilde flora

2.2.1.1. Selecting the vegetal material

2.2.1.2. Javellization of the sections

2.2.1.3. Colouring of the sections

2.2.1.4. Mounting of the sections

2.3. The study of some morphological parameters

2.4. The phytochemical study of the vegetal material samples included in the study

2.4.1. *Extraction methods used*

2.4.1.1. Obtaining methanolic extracts (DER=3:100g/mL)

2.4.1.2. Obtaining volatile fractions

2.4.2. *Qualitative chemical analysis*

2.4.2.1. TLC analysis of the flavons

2.4.2.2. TLC analysis of the polyphenolcharboxylic acids

2.4.2.3. TLC analysis of nonvolatile monoterpens (iridoids)

2.4.2.4. TLC analysis of triterpens

2.4.3. *Quantitative chemical analysis*

2.4.3.1. Spectrophotometric determinations

2.4.3.1.1. Spectrophotometric determination of the flavon

The general part, ***General knowledge regarding the chemistry of the iridoids and present knowledge referring to the species studied,*** is made up two subchapters:

- *general considerations regarding the iridoidic group and*
- *present knowledge regardinf the studied species*

The personal part starts with chapter II, ***Material, methods and working techniques***, which refers to the **histo-anatomical analysis** achieved by classical microscopy of the species *Ajuga reptans* L. and *Ajuga genevensis* L., **biometric analysis** performed on the two *Ajuga* species, on *Betonica officinalis* L. and on two verbascum species, *Verbascum phlomoides* L. and *Verbascum thapsiforme* Schrad. The **phytochemical analysis** was performed on the vegetal material prelevated from the spontaneous flora, made up of the two species of *Ajuga* and *Verbscum*, of *Betonica officinalis* L., *Galium verum* L. and *Galium album* Mill. For each species we analysed more samples from different locations, the prelevations and the phytochemical analysis being performed over the years 2010, 2011, 2012. This was constituted of **thin layer chromatography** (TLC) for the most important groups of active principles, which were completed by **spectrophotometric determinations** of the iridoids, flavonoids and polyphenolcharboxylic acids. The study of the polyphenolic fraction was completed by **the high performance liquid chromatography** (HPLC), and in the case of the two *Ajuga* species, after water vapour distillation of the fresh vegetal material, we identified volatile compounds by **gas-chromatography coupled with mass spectrometry (**GC-MS).

Chapter III, ***Contributions to the biological and phytochemical study of some Ajuga reptans L. and Ajuga genevensis L. individuals prelevated from the spontaneous***

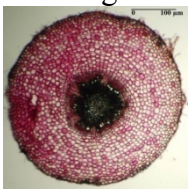
flora and from culture presents the researches on bugle and blue bugleweed plants and the results obtained.

***Ajuga reptans* L.**

***Ajuga genevensis* L.**

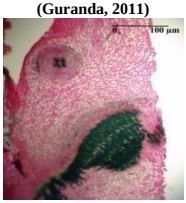
Fig 1. Natural populations of *Ajuga reptans* L. and *Ajuga genevensis* L. (Original Photo)

The histo-anatomic study aimed to highlight the characteristic elements in the cross sections achieved in the roots and rhizomes, stems and leaves of the two *Ajuga* species, as shown by the images in fig. 2,3,4.

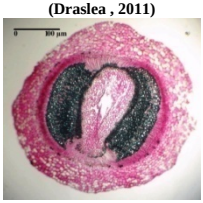


***Ajuga reptans* L.–root (Guranda, 2011)**

***Ajuga genevensis* L.–root (Draslea , 2011)**

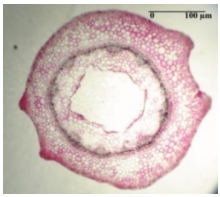


***Ajuga reptans* L.–rhizome (Guranda, 2011)**

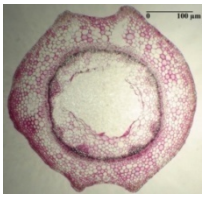


***Ajuga genevensis* L.–rhizome (Draslea, 2011)**

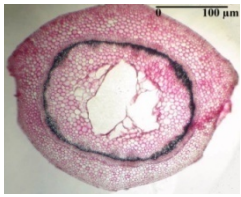
Fig 2. Cross section through the root and rhizome of *Ajuga reptans* L. and *Ajuga genevensis* L.



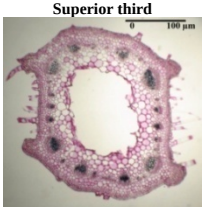
***Ajuga reptans* L.:**
Superior third



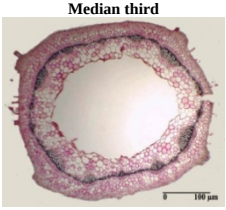
***Ajuga reptans* L.:**
Median third



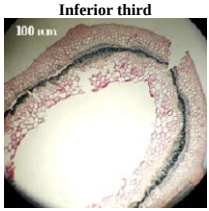
***Ajuga reptans* L.:**
Inferior third



***Ajuga genevensis* L.:**
Superior third

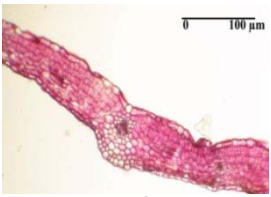


***Ajuga genevensis* L.:**
Median third

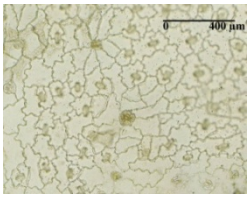


***Ajuga genevensis* L.:**
Inferior third

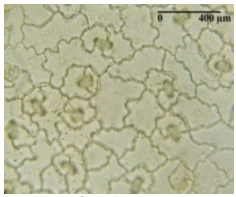
Fig. 3. Cross section through the superior median and inferior third of the aerial stem of *Ajuga reptans* L. and *Ajuga genevensis* L.



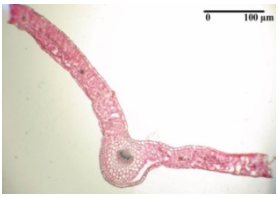
***Ajuga reptans* L.–leaf**



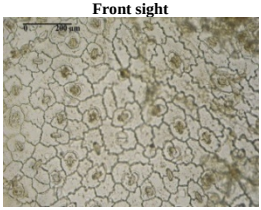
Superior epidermis
Front sight



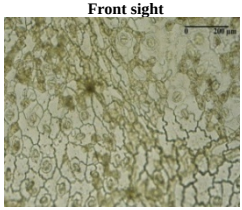
Inferior epidermis
Front sight



***Ajuga genevensis* L.–leaf**



Superior epidermis
Front sight



Inferior epidermis
Front sight

Fig. 4. Cross section through the *Ajuga reptans* L. and *Ajuga genevensis* L. leaf

With the **morphological analysis,** we aimed the branching number for each individual basic rosette of individuals of

Ajuga reptans (bugle) prelevated at Guranda, compared with those of *Ajuga genvensis* (blue bugleweed) harvested from Draslea, on May, 20, 2011(fig.5).

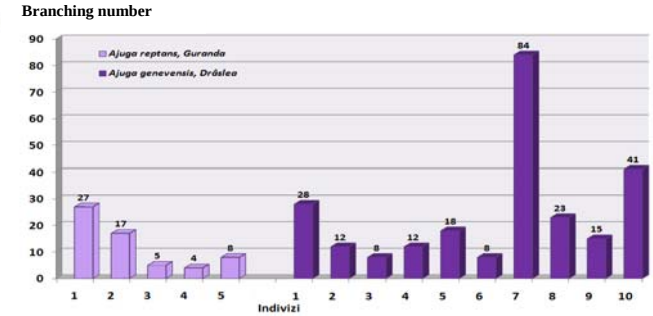


Fig. 5. The variation of the branching number in case of *Ajuga reptans* L. and *Ajuga genevensis* L. individuals from the spontaneous flora

Weighing the fresh mass of the individuals analyzed above, we obtained values between 12.64 and 236.93g for *Ajuga reptans* compared to 31.05 and 337.98g for *Ajuga genevensis* (fig. 6).

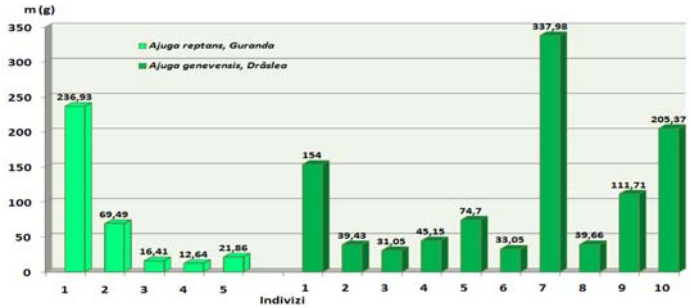


Fig. 6. Variations in weight from the spontaneous flora *Ajuga reptans* L. si *Ajuga genevensis* L. individuals

It results that *Ajuga genevensis* offers individuals with a greater number of branchings and richer fresh weight than *Ajuga reptans*, due to the fact that blue bugleweed is a less demanding plant regarding soil and climate.

Taking into account the fact that in 2009 samples of *Ajuga reptans* prelevated at Guranda were transfered into the experimental field of CCB "Stejarul" Piatra Neamt, so that in May 2010, 2011 and 2012 we could harvest individuals that underwent the same measurements, the results obtained are in fig. 7 and 8.

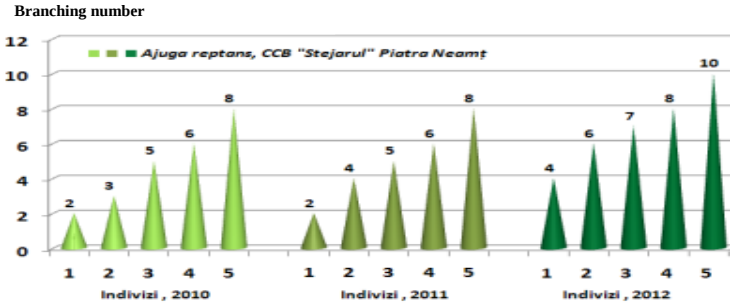


Fig. 7. The variation of the branching number of cultivated *Ajuga reptans* L. individuals

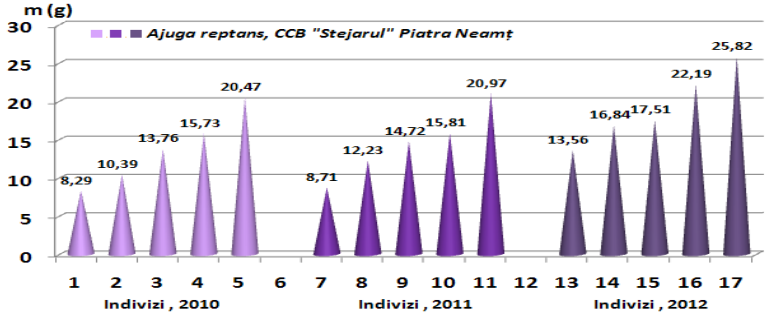


Fig. 8. Variations in weight of cultivated *Ajuga reptans* L. individuals

It is interesting that both the branching number/ individual and the fresh weight, results obtained in 2010 and 2011 are very similar, while, in the third culture year both the branching number and the determined fresh weight increased.

The phytochemical analysis started with TLC for the polyohencharboxylic acids (fig.9) and the flavonoids (fig.10.).

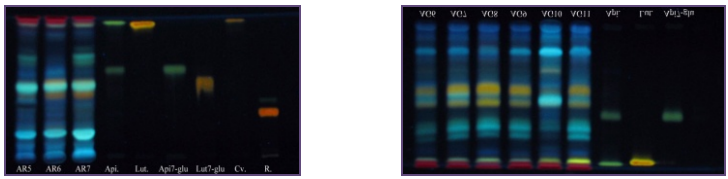


Fig. 9. TLC for flavonoids of *Ajuga* vegetal material extracted in 2011

***Ajuga* reptan samples:** AR5=Guranda, AR6=Bicaz, AR7=Grozăveşti; **Probe*Ajuga genevensis*:**AG6=Mascateni, AG7=Drâslea(fl.mov), AG8=Drâslea(fl.roz), AG9=Stauceni, AG10=Baisa, AG11=Grozăveşti

Flavon standards:: apigenol(Api), luteolin (Lut), apigenin-7-O-glucoside (Api7-glu), luteolin 7-O-glucoside(Lut7-glu), cvercetol (Cv), rutozid (R)

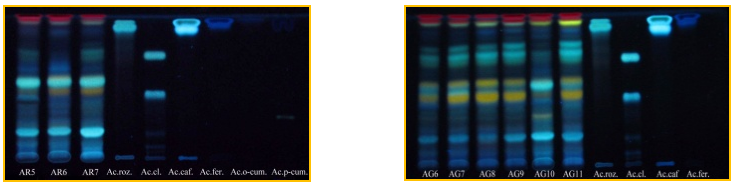


Fig. 10. TLC for polyphenolcharboxylic acids in *Ajuga reptans* and *Ajuga genevensis* individuals prelevated in 2011

***Ajuga reptans* samples:** AR5=Guranda, AR6=Bicaz, AR7=Grozăveşti; **Probe*Ajuga genevensis*:**AG6=Mascateni, AG7=Drâslea(fl.mov), AG8=Drâslea(fl.roz), AG9=Stauceni, AG10=Baisa, AG11=Grozăveşti

Polyphenolcharboxylic acids standards: rozmarinic acid (Ac.roz.), clorogenic acid (Ac.cl.), cafeic acid (Ac.caf.), ferulic acid (Ac.fer.), o-cumaric (Ac.o-c.) acid, p-cumaric acid (Ac.p-c.)

As we may notice, the chromatographic pictures obtained for the extracts of three *Ajuga reptans* samples and six *Ajuga genevensis*, show the presence of a certain intraspecific chemical variability in case of *Ajuga genevensis* (see sample AG10), but especially interspecific.

On the other hand, TLC of iridoids shows the presence of three components of this type in *Ajuga reptans*(fig. 11), compared to only two in *Ajuga genevensis* (excepting sample AG7).

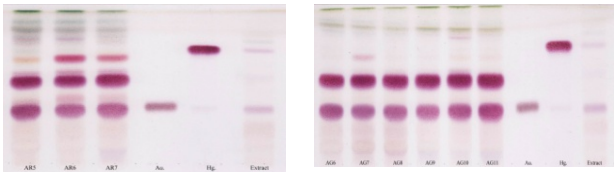


Fig. 11. TLC for iridoids in *Ajuga* extracts in 2011

***Ajuga reptans* samples:** AR5=Guranda, AR6=Bicaz, AR7=Grozăveşti; **Probe*Ajuga genevensis*:**AG6=Mascateni, AG7=Drâslea(fl.mov), AG8=Drâslea(fl.roz), AG9=Stauceni, AG10=Baisa, AG11=Grozăveşti

Iridoid standards:aucubosid (Au), extract *Harpagophytum procumbens* commercial (extract), 8-O-acetil harpagid (Hg)

Making the spectrophotometric determinations for polyphenols, then followed by the identification by means of HPLC of some components, compared to a series of sandards, we found the presence in the analysed vegetal material of some small quantities of chlorogenic, caffeic, p-cumaric acids, of apigenol and luteolin-7-O-glucosides. Comparing the spectrophotometric values with the ones obtained by HPLC, we noticed that the majority of the polyphenolic components could not be identified becuase of lack of adequate standards, as we can see in fig. 12 and 13.

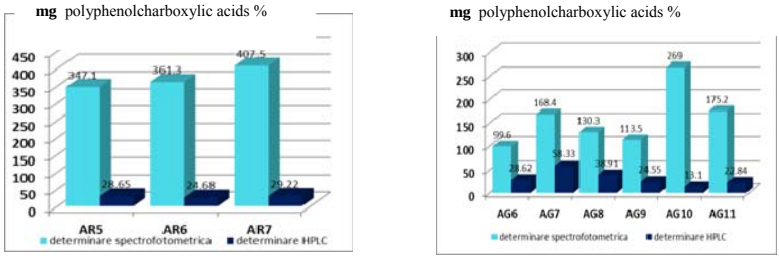


Fig. 12. **Comparing the values obtained in 2011 by spectrophotometric dosing and HPLC analysis for polyphenolic acids in *Ajuga reptans* and *Ajuga genevensis* individuals**

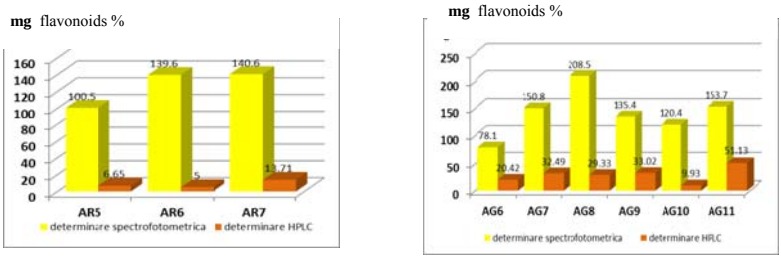


Fig. 13. **Comparing the values obtained in 2011 by spectrophotometric dosing and HPLC analysis for flavonoids in *Ajuga reptans* and *Ajuga genevensis* individuals**

Trying to quantify the polyphenolcharboxylic acids and the flavonoids from the sample of *Ajuga reptans* (bugle) prelevated in 2012 at Guranda, compared to the one in the third year of culture at Piatra Neamt (initially transfered from

Guranda), we again resorted to the spectrometric determination *versus* HPLC. The results are in fig. 14 and 15.

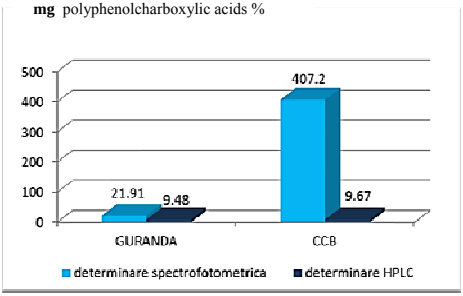


Fig. 14. **Comparing the values obtained in 2012 by spectrophotometric dosing and HPLC analysis for polyphenolic acids in *Ajuga reptans* individuals – spontaneous and cultivated**

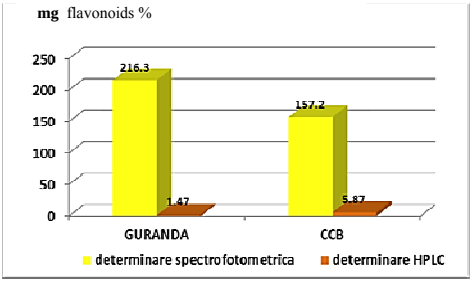


Fig. 15. **Comparing the values obtained in 2012 by spectrophotometric dosing and HPLC analysis for flavonoids in *Ajuga reptans* individuals – spontaneous and cultivated**

Comparing the values obtained in the spectrophotometric dosing for polyphenolcharboxylic acids in the spontaneous and cultivated product, we notice a very low content of such substances for the Guranda sample and 20 times higher for the one of Piatra Neamt (CCB "Stejarul").

As to the flavonoids, the Guranda sample proves to be richer, and the one of Piatra Neamt has a lower content by approximately 30%. This might be explained by the fact that the exposition to light was much higher at Guranda, fact that

favoured the flavonoid synthesis, while at Piatra Neamt (CCB "Stejarul") the plant was less exposed to sun.

Yet, on the other hand, we notice that by HPLC analysis we could identify and quantify very few of the components, the majority of which remaining unidentified because of lack of adequate standards.

Another group of components monitored in the *Ajuga* species was made up of that of iridoids, when we determined for *Ajuga reptans* values between 0.4335 and 0.617g E aucuboside, while *Ajuga genevensis* has a more reduced content (0.1243–0.3997g), the results obtained being in fig. 16.

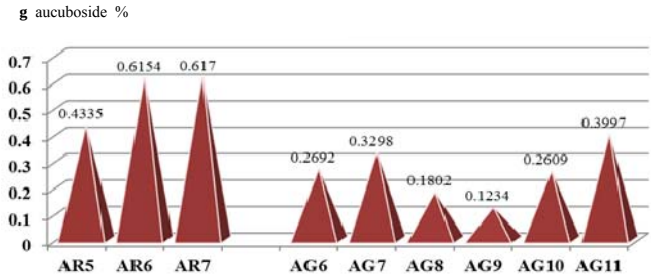


Fig. 16. The graphical representation of the iridoid content determined in the samples of *Ajuga* – 2011

Ajuga reptans* samples:** AR5=Guranda, AR6=Bicaz, AR7=Grozavesti; **Probe *Ajuga genevensis: AG6=Mascateni, AG7=Draslea(fl.mov), AG8=Draslea(fl.roz), AG9=Stauceni, AG10=Baisa, AG11=Grozavesti

Finally, we made **GC-MS** analysis of the volatile oil distilled from the fresh vegetal material of the two *Ajuga* species and noticed that, in both cases, the majority is acetophenone, followed by indan, 2-metylbenzaldehide, tetrahydrodicylopentadiene and D-carvon, the last not being present in *Ajuga genevensis*. In fig. 17 and we give the chemical composition of the four bugle and blue bugleweed populations.

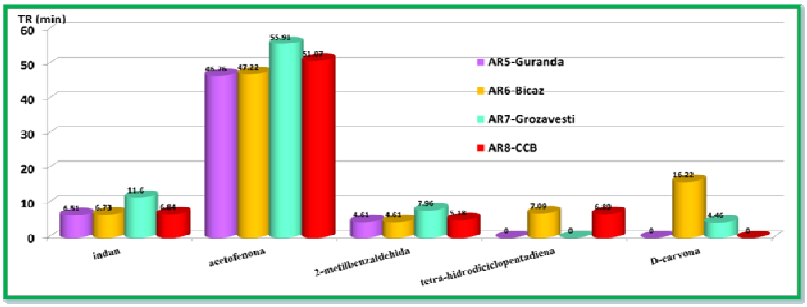


Fig. 17. The variation of the content of indan, acetophenone, metylbenzaldehide, tetra-hydrodicyclopentadiene and D-carvon in the volatile oil of the *Ajuga reptans* individuals

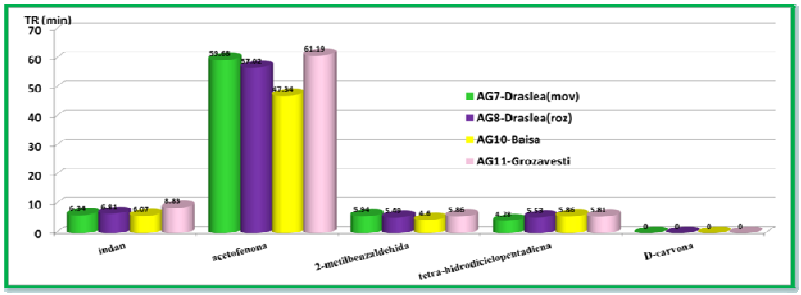


Fig. 18. The variation of the content of indan, acetophenone, metylbenzaldehide, tetra-hydrodicyclopentadiene and D-carvon in the volatileoil of *Ajuga genevensis* individuals

Chapter IV refers to ***Researches regarding the biology and phytochemistry of some natural populations of Galium verum L. and Galium album Mill..***

The yellow fairies represent plants frequently used in European traditional medicine and the species is only sometimes replaced by *Galium album* (fig.19).

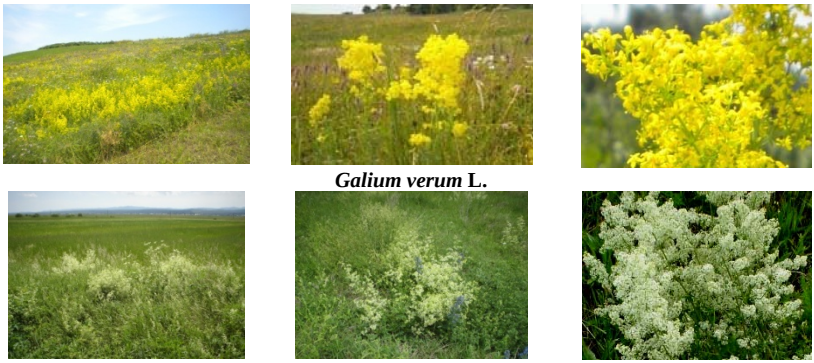


Fig. 19. Natural populations of *Galium verum* L. and *Galium album* Mill. (Original Photo)

TLC performed as in the previous chapter to highlight the polyphenolcharboxylic acids and flavonoids (fig.20) proved the existence of some chemical variations, both intra- (compare samples GV7 to GV8) and interspecific (compare GV7 to GA2).

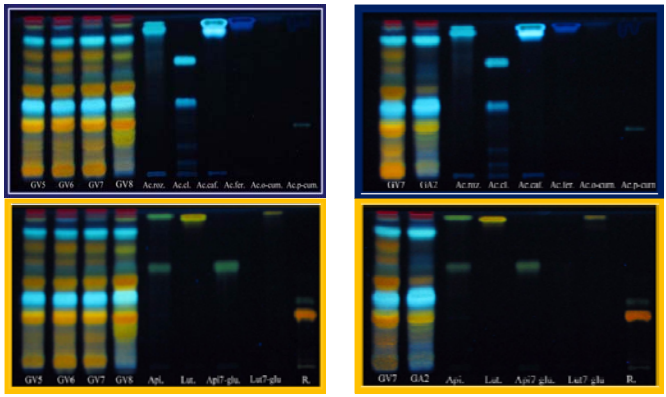


Fig. 20. The thin layer chromatograms for flavons and polyphenolcharboxylic acids from *Galium verum* and *Galium album* (2011) samples

***Galium verum* samples: GV5=Tarzia, GV6=Radaseni, GV7= Tolici, GV8=Negresti; Probe *Galium album*:** GA2=Tolici
Polyphenolcharboxylic acids standards: rozmarinic acid (Ac.roz.), clorogenic acid (Ac.cl.), cafeic acid (Ac.caf), ferulic acid (Ac.fer), o-cumaric acid (Ac.o-cum.), p-cumaric acid (Ac.p-cum)
Flavon standards: apigenol (Api), luteolin (Lut), apigenin-7-O-glucoside (Api7-glu), luteolin 7-O-glucoside (Lut7-glu), rutosid (R)
Iridoid standards: aucubosid (Au.), extract *Harpagophytum procumbens* commercial (extract.), 8-O-acetil harpagid (Hg.)

Regarding the iridoidic fraction (fig.21), this seems to have an almost similar composition for the plants of the two species.

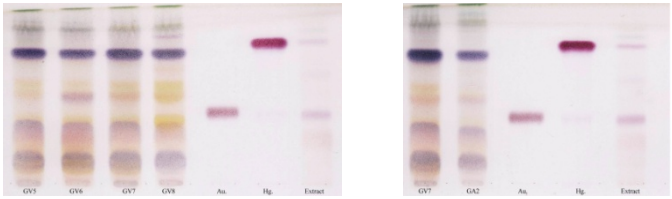


Fig. 21. The thin layer chromatograms for iridoids of *Galium verum* and *Galium album* samples (2011)

The spectrophotometric dosing of the three groups of monitored active principles (iridoids, polyphenolcharboxylic acids, flavonoids) ended up in the values shown by fig. 22.

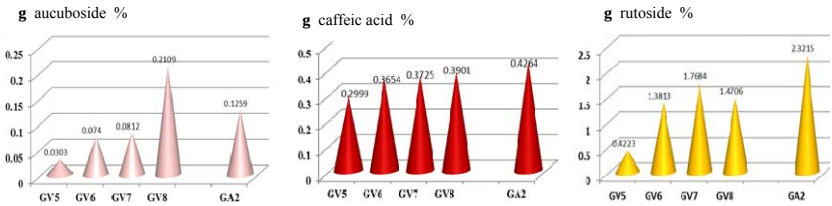


Fig. 22. The variation of the content of iridoids and polyphenols determined in *Galium verum* L. and *Galium album* Mill. populations harvested in 2011

We notice that regarding the iridoids, the content varies, even intraspecifically, very much, from 0.0303 g to 0.2109 g for *Ajuga reptans,* in case of the same from Tarzia being registered the lowest values of flavonoids (0.4223g rutoside) and polyphenolic acids, respectively (0.2999g). The best population, from the chemical point of view, seems to be the Negresti bugle. As to the Tolici *Galium album* population, this seems to be qualitatively richer in active principles than the yellow fairies harvested in the same location.

The HPLC analysis proved the presence in the analyzed material of chlorogenic acid, rutoside, luteolin and luteolin7-O-glucoside. The quantities of chlorogenic acid and rutoside identified with this occasion are in fig. 23.

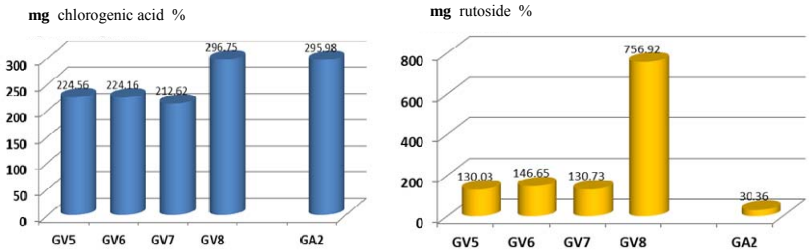


Fig. 23. The contents of chlorogenic acid and rutoside identified by HPLC in *Galium* verum and G. *album* in 2011

If the chlorogenic acid proves to be in comparable quantities in the samples of *Galium verum* from Tarzia, Radaseni and Tolici, for the Negresti sample, the quantity of this compound is higher. Speaking about rutoside, we notice a quantitative similarity for the first three samples, the same sample of Negresti being up to five times richer. Yet, in *Galium album* the quantity of chlorogenic acid is higher and that of rutoside is very low.

Chapter V presents ***The biological and phytochemical study of the Betonica officinalis L. species harvested in the North-Eastern part od Moldavia.***

*Betonica officinalis*L. (fig.24) known in literature under the name of *Stachys officinalis* L. is a lamiaceae little studied in our country.

Fig. 24. The aspect of *Betonica officinalis* L. Plants (Original Photo)

The TLC analyses for polyphenolcharboxylic acids and flavonoids (fig. 25) show a reduced intraspecific chemical variability, fact that confirms itself for the iridoidic fraction, too (fig.26).

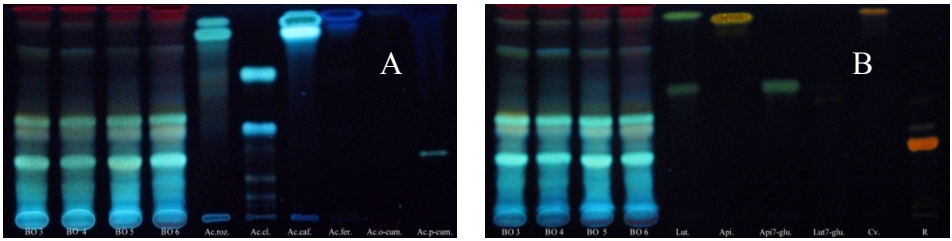


Fig. 25. Thin layer chromatograms for polyphenolcharboxylic acids (A) and flavonoids (B) in *Betonica officinalis* L. Samples (2011)

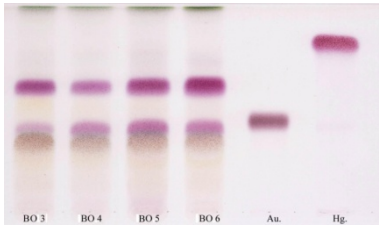


Fig. 26. Thin layer chromatograms for iridoids in *Betonica officinalis* L. Samples (2011)

***Betonica officinalis* samples:** BO3= Tarzia, BO4= Tolici, BO5= Frumosu, BO6= Calinesti
Flavon standards: apigenol(Api), luteolina(Lut), apigenin-7-O-glucoside (Api7-glu), luteolin 7-O-glucoside (Lut7-glu), rutosid(R)
Polyphenolcharboxylic acids standards: acid rozmarinic(Ac.roz.), acid clorogenic(Ac.cl.), acid cafeic (Ac.caf), acid ferulic(Ac.fer), acid o-cumaric(Ac.o-cum.), acid p-cumaric (Ac.p-cum); **Iridoids standards:** aucubosid (Au.), 8-O-acetilgarpagid (Hg.)

The spectrophotometric determinations made for flavonoids, polyphenolcharboxylic acids and total polyphenols show the clear existence of an intraspecific chemical variability, as shown in fig. 27.

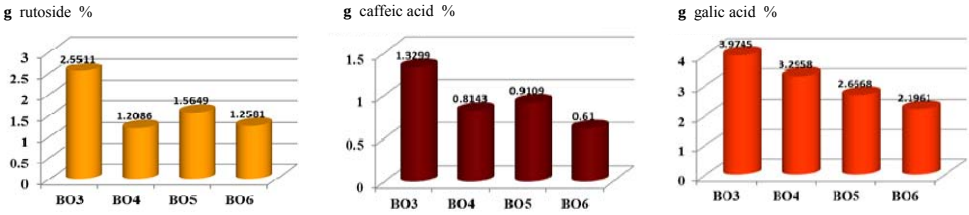


Fig. 27. The variation of the flavonoid, polyphenolchaboxylic acids and total polyphenols content in *Betonica* samples harvested in 2011

Continuing the phytochemical analysis by an HPLC study, we identified and quantified rosmarinic, chlorogenic, caffeic, p-cumaric acids, luteolin and apigenol-7-O-glucoside, part of these compounds being graphically expressed in fig. 28.

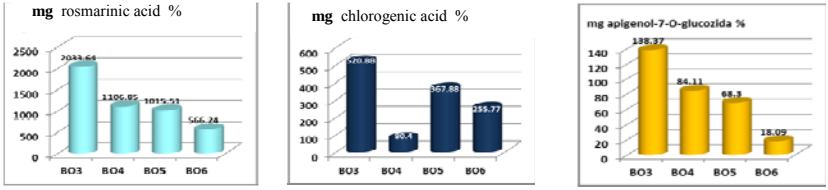


Fig. 28. The variation of the rosmarinic, chlorogenic acids and apigenol 7-O-glucoside content in *Betonica* extracts analysed by HPLC in 2011

The last two species studied are presented in chapter VI, with the title ***Researches regarding two verbascum species: Verbascum phlomides L. andi Verbascum thapsiforme Schrad.: biometric and phytochemical studies.***

From the point of view of the general aspect, the two species differ rather much, as shown in figures 29a and 29b.

Fig. 29a. The aspect of some *Verbascum phlomoides* L. individuals (Original Photo)

Fig. 29b. The aspect of some *Verbascum thapsiforme* Schrad. idividuals (Original Photo)

TLC allowed the identification of same components of the polyphenolcharboxylic acid type and less of flavonoids (fig.30), as well as of some iridoids (fig.31).

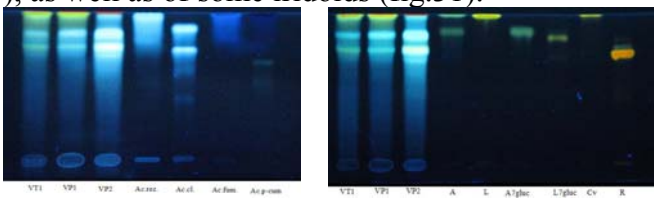


Fig 30. The thin layer chromatogram for polyphenolcharboxylic acids and flavons in *Verbascum phlomoides* and *Verbascum thapsiforme* prelevated in 2010

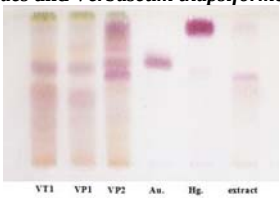


Fig. 31. The thinlayer chromatogram for iridoids in *Verbascum phlomoides* and *Verbascum thapsiforme* plants prelevated in 2010

Samples: VT1= *V. thapsiforme* Tolici; VP1= *V.phlomoides* Savinesti; VP2= *V.phlomoides* Frumosu
Etaloane acizi polifenolcarboxilici: acid rozmarinic(Ac.roz.), acid clorogenic(Ac.cl.), acid cafeic (Ac.caf), acid ferulic(Ac.fer), acid p-cumaric (Ac.p-cum)
Flavon standards: apigenol(A), luteolin(L), apigenin-7-O-glucoside (Ap7glu), luteolin 7-O-glucoside (L7glu), cvercetol (Cv), rutosid (R)
Iridoid standards: aucubozida (Au.), 8-O-acetilgarpagida (Hg), extract *Harpagophytum procumbens* commercial (extract)

Repeating, in 2011, the chromatographic analysis on such organs as: flowers, leaves and roots, the chromatographic pictures were different: in case of the flowers, along with one dominant polyphenolcharboxylic acid we also identified a rich flavonoidic component (fig. 32), the leaves and roots seeming to lack flavonoidic pigments.

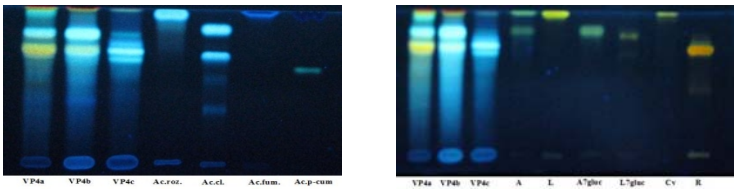


Fig. 32. Thin layer chromatogram for the polyphenolcharboxylic acids and flavons in the organs of *Verbascum phlomoides* prelevated in 2011

Samples *Verbascum phlomoides* - Frumosu: VP4a=flowers, VP4b=26 leaves, VP4c=roots

Regarding the iridoidic fraction of the different organs (fig. 33), we notice that this is well represented especially in flowers and roots, but different from one organ to another.

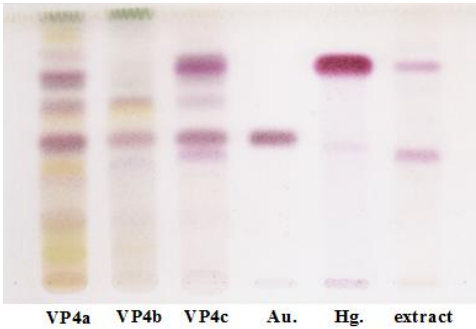


Fig. 33. Thin layer chromatogram for iridoids in the organs of *Verbascum phlomoides* prelevated in 2011

Samples *Verbascum phlomoides* - Frumosu: VP4a=flowers, VP4b=26 leaves, VP4c=roots

Achieving spectrophotometry on extracts obtained from the three types of organs, we noticed the existence of a rather important variation of polyphenols from one organ to another.

Regarding the polyphenolcharboxylic acids, the roots have the lowest content, while the leaves contain a great quantity of these components (fig.34).

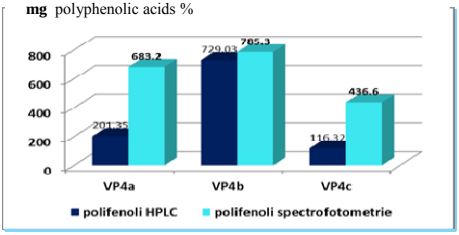


Fig. 34. The content of spectrophotometrically deternived polyphenolic acids compared to the quantification by HPLC for flowers, leaves and roots of *Verbascum phlomoides*

Completing the spectrophotometric dosings with HPLC analysis, we could establish the toatl level of the identified polyphenolic acids (made up of rosmarinic, chlorogenic, caffeic, p-cumaric acids) in the three types of vegetal products, as shown in fig. 34.

As to the flavonoids, comparing the values registered by the spectrophotometric dosing to those obtained by the HPLC analysis (the identified flavonoids being: luteolin, apigenol and rutoside), we notice that in flowers and leaves the content in such components is close, while in the roots it is lower.

On the other hand, the roots contain neither ritoside nor luteolin or apigenol, while in the flowers these represent aproximately 35%, and in the leaves, one eighth of the total flavonoidic pigments (fig.35).

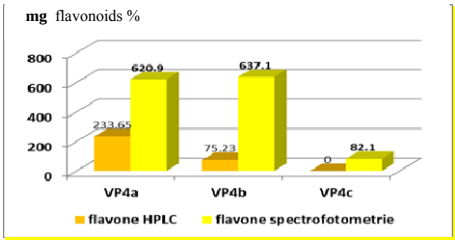


Fig. 35. The content of flavonoids determined spectrometrically compared to the quantification by HPLC for flowers, leaves and roots of *Verbascum phlomoides*

Samples *Verbascum phlomoides* - Frumosu: VP4a=flowers, VP4b=27 leaves, VP4c=roots

The thesis ends with, ***Conclusions.***

Considering the results obtained in our research, we may formulate, depending on the investigated species, the following conclusions for:

***Ajuga reptans* L. / *Ajuga genevensis* L.**

- The histo-anatomical study showed the existence of some similarities at the level of the root and rhizome structure, while
  - at the level of the stem the margins of the cross section is square shaped in *Ajuga genevensis* and circular in *Ajuga reptans*,
  - both present peritectors, formed of two to seven cells and secretors with unicell pedicell and bicell gland,
  - at the level of the leaf, the stomata are of the diacitic type, present in both epiderms,
  - from the biometrical point of view, the *Ajuga genevensis* plants are better developed, being less demanding to the soil offer.
- On the phytochemical level there are:
  - an interspecific variability,
  - an intraspecific variability,
  - a reduced intrapopulational variability (pink/mauve flowers),
- *Ajuga reptans* is richer in active principles,
  - it contains chlorogenic, caffeic, p-cumaric acids, apigenol and luteolin7-O-glucoside,
  - the volatile oil mainly contains acetophenone and indan, metylbenzaldehide, tetracyclopentadiene and D-carvon (present only in *Ajuga reptans*)

- The individuals of *Ajuga reptans* were transferred into culture at "Stejarul" Piatra Neamt and maintained their biosynthetical capacity.

***Galium verum* L. / *Galium album* Mill.**

- the inter- and intraspecifc chemical variability is present,
- the yellow fairies are richer in active principles of the iridoidic and polyphenolic types,
- they contain chlorogenic acid, rutoside, luteolin and luteolin-7-O-glucoside

***Betonica officinalis* L.**

- the morphological evaluation allowed the identification of some variations in the normal limits of some parameters,
- it presents intraspecific variability, that may be highlighted by the spectrophotometric dosing and the HLPC analysis,
- it contains rosmarinic, chlorogenic, caffeic, p-cumaric acids, luteolin and apigenol-7-O-glucoside.

***Verbascum phlomoides* L. / *Verbascum thapsiforme* Schrad.**

- their general aspect is morphologically different,
- the interspecific variability is present as well as the intraspecific one, yet the intrapopulational variability is very reduced,

- the climatic conditions of the prelevation year reflect the accumulation of the active principles,
- the spectrum of the components and the quantity in which they are present in different plant organs vary very much,
- they contain rosmarinic, chlorogenic, caffeic, p-cumaric acids, luteolin, apigenol and rutoside.

Originality degree

- derives from the investigation of less studied spontaneous indigenous species,
- aims pharmaco-botanical, chemical and biological aspects,
- contributes to the broadening of knowledge linked with the seven investigated species and with the correlations existing between the pedoclimatic conditions and the aspects of morphology and chemical composition,
- the project goes beyond the purely theoretical importance having a practical finality by offering the specialists some data useful to obtain some medicine preparations or some food supplements, and by the transfer of the *Ajuga reptans* L. species from the wild flora into culture and the monitoring the cultivated plant from the point of view of its viability and biosynthetic capacity of some important biological principles.

The thesis has 220 pages, a bibliography of 200 titles and is illustrated by 39 tables and 145 figures.

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