

ABSTRACT

The present thesis proposes to study medicinal herbs with hepatic- gall bladder action, plants which were cultivated in the pedoclimatic conditions of the Tecuci area. Owing to the fact that most of the diseases encountered in this area are of hepatic-bladder influence, we have introduced in the study five of the most efficient medicinal herbs, observing the accumulation of active principles, and with the purpose of inserting them in the phytotherapy of such affections. Although only small areas are currently cultivated with medicinal herbs, I will try and determine the extent of these cultures as well as the possibility of introducing them in various phytotherapeutic procedures.

My desire is to foster in my students a certain degree of confidence in using phytotherapy to help themselves as well as their families, avoiding at the same time the toxicity of chemical drugs.

The doctoral thesis is structured in 9 chapters, spanning over 279 pages, with 69 tables and 119 figures.

The chapters are organized in two parts. The first part represents a synthesis of the bibliographic data essential in the knowledge of liver and gall bladder diseases, their phytotherapy and the homeopathy of digestive diseases using the 13 plants initially introduced in the study (bearing in mind the fact that homeopathy doesn't treat diseases, but the sick persons). The results of personal research focusing on the subject of the doctoral thesis are presented in the second part. This part sums up 148 pages, 65 tables and 82 figures.

The experimental lot was placed at the outskirts of Tecuci and comprised cultures of: *Cynara scolymus*, *Silybum marianum*, *Calendula officinalis*, *Hyssopus officinalis* and *Mentha piperita*.

The culture was ecological (no chemical fertilizers were administered; no disease or pests treatments were carried out).

Climatic conditions in the years of experimenting were very different. The years 2006 and 2008 were particularly favorable to the culture of medicinal herbs, while the year 2007, with its drought, seriously diminished the quantity and the quality of the production.

The five herbs have been observed from a phenological, biometrical and biochemical perspective. Biochemical determinations destined to reveal the accumulation of active substances were carried out at the PlantExtrakt firm in Cluj County. These determinations had as a purpose the establishment of flavonoids' concentration accumulated in ecological cultures, within the pedoclimatic conditions from South Moldavia. Very good results were recorded in the cultures of *Cynara scolymus*, *Silybum marianum*, *Calendula officinalis*, which have managed to accumulate active substances, above the minimum registered value both in the Romanian and European Pharmacopeea.

Cynara scolymus

Observations and research was carried out in the dynamics of the vegetation, monitoring its growth and development. In first 30 days from emergence measurements were run every 3, 9, 15, 20, 30 days.

Biometrical tests carried out in the laboratory were focused on the leaves, the whole plants and the weight of the fresh vegetal mass. The leaves and flowers have larger parameters of length and width in 2008, owing to the better climatic conditions, as compared to the previous years.

If we add to these parameters the weight and the number of the leaves from the plant, we can speak of a bigger production of vegetative mass per hectare in the year 2008 as compared to 2006 or 2007.

In the vegetation period (April-October, November), plants grow and develop. The recorded values show an evolution in plants height, from 70 cm in May up to 150-180 cm in September. The number of leaves registers a certain growth in July only to diminish in September with almost 50 %. Inflorescence in July is up to 50 % less encountered than in August and September.

The surface of the *Cynara* leaves varied from 1710 cm² to 8700 cm² in 2008. From the weighting carried out on the 15th of May, of July and the 15th of September in 2008, has resulted a production of 10.400 kg/ha in May, 17.200 kg/ha in July and 20.000 kg/ha in September. Thus there is indication of a massive growth of the vegetal mass in July, August and September, with a double production in the latter as compared to that from May.

The spectrophotometrical analysis of the assimilating pigments content from the *Cynara scolymus* leaves reveals that, in May, the concentration in „a” chlorophyll was of 15,40 mg %, with a decrease of this parameter during vegetation, thus reaching a difference of more than 4% as compared to May. However, there is evidence of an increase in the content of „b” chlorophyll, to such an extent that, by the end of the vegetation the growth is as much as 4% bigger than in May. In general, even if chlorophyll „ b” increases in quantity, on the whole, the total amount of chlorophyll decreases during vegetation.

The relation between the two types of chlorophyll decreases as well with almost 8%. The identification of specific flavonoids through chromatography on a slim layer of The analyzed *Cynara scolymus* leaves revealed the separation of a set of polyphenols-derived from cafeic acid and flavonoids, out of which, by comparison to ethanol, clorogenic acid was identified.

Following the spectrophotometrical dosing of the flavonoids, as revealed in rutozid, a value of $3,03 \pm 0,15\%$ was obtained. This is a value of approximately 8,5 times bigger than the minimum registered for this plant in the Romanian Pharmacopeea the 10th edition (min. 0.35%).

This proves that the area of Tecuci is favoring the accumulation of flavonoids in *Cynara scolymus*.

Silybum marianum

The culture was established in the spring of 2006, by sowing, after the germination power of the seeds had been tested.

The biological material selected was biometrically tested in the laboratory, paying attention to the height, the number of leaves and the number of inflorescences on the plant, as well as the weight of the fresh vegetal mass. In the first month of vegetation, the growth was massive, the plant reaching a length four times its size. The number of leaves from the plant doubled too. In July we could notice a more reduced height growth, with a tripled number of leaves however.

The production in May was below average registered value, while in July it was a bit more than half of it. At the end of the vegetation period however, in September, the production was twice and a half larger than the average.

The chromatograms carried out for the identification of specific flavonoids reveal in the sample the stripes characteristic to silylline, taxofiline and silicristine, according to the description in the European Pharmacopeea. The chromatography on a slim layer separated the specific silimarins: silylline, taxofiline and and silicristine.

Following the spectrophotometrical dosing of the silimarins expressed in silylline, a value of $1,74 \pm 0,07$ % was obtained.

According to the German Pharmacopeea, Xth edition, the minimum quality condition is of 1% silimarin expressed in silylline.

As a result, the pedoclimatic conditions in South Moldavia favor the accumulation of flavonoids, since average values of more than 0.74% were recorded.

Flavonoids, the active substance present in milk thistle seeds, favors the regeneration of hepatic cells and increase the organism's capacity, especially the liver's, to protect itself from infections, which leads to its using with very good results in treatments against viral hepatitis of the A, B and C type.

It is also recommended in the treatment of icterus and hepatic insufficiency, in which case silibinine, another substance from the plant, prevents the accumulation of toxins in the liver, thus helping the hepatic cells and stimulating the elimination of toxic compounds from the hepatic cells.

Silimarin stimulates the regeneration of the hepatocyte (liver cell), the emergence and development of new liver cells, sustaining the synthesis of the proteins. It protects the integrity of the hepatocyte's cell membrane by preventing hepatic-toxic substances from getting into liver cells (ethylic alcohol, toxic chemical substances, drugs having a toxic effect on the liver, toxins resulted after food processing, etc.).

At the same time, silimarin reduces the risk of developing gallstones and sustains the digestion, by decongesting and eliminating the bladder.

Calendula officinalis

The biochemical tests have determined the content in assimilating pigments, the saponines and the spectrophotometrical dosing of the flavonoids as they are expressed in rutozid. The obtained saponine has a dirty white-yellowish color, with a pH = 4,5 – 5 when dissolved in water, and a very abundant foam when stirred, that lasts for about two hours.

The identification chromatogram of the specific flavonoids, carried out through chromatography on a slim layer shows that the strips of separated compounds are similar in the two samples (flowers harvested in August and September), with the strips in sample A more intense than those in sample B. One can also notice strips specific to saponines around the level of the escine.

After comparing reference values of galic and escin acid norms with the values obtained from separated compounds in the samples, we notice the same values, thus proving the presence of the saponines.

The chromatograms used for the identification of specific flavonoids, before and after spraying with NTS, indicate strips of separated compounds similar in the two samples, with the strips more intense in sample a and less so in sample b. One may notice strips right in front of the rutin and the chlorogenic acid. The comparative analysis of the samples in relation with rutin and chlorogenic acid norms confirmed the presence, in sample a, of chlorogenic acid and rutin. This confirmation is based on the comparison of R_f values of the norm with those of the separated compound from the samples, and more precisely on comparing the aspect of the norm's strip with that of the compound separated in the samples.

The yellow-brown strips correspond to the flavonoids, while the blue ones correspond to the poliphenocarbolic derivatives.

Following the spectrophotometrical dosing of the flavonoids as expressed in rutozid, a value of $0,60 \pm 0,03$ % was obtained.

According to the European Pharmacopoeia, the minimum quality condition is of a total of 0,4% flavonoids expressed in the hiperozide.

The concentration of flavonoids in the samples is up to 50 % bigger than the minimum value, which shows a stronger therapeutic capacity.

Hyssopus officinalis

The Hyssop culture started off on the 8th of April 2006, by direct sowing in the field.

We notice that, in 2008 has been recorded the largest production of fresh herba, with an increase of almost 60% as compared to 2006 and over 40% in comparison with 2007.

In this case as well, the high temperatures during summer, combined with the lack of rainfall in 2006, and especially in 2007, have led to modest vegetal growth, as compared to the year 2008.

For the dry herba the production report is the same, reaching a top level in 2008 (3125 kg/ha) and a low level in 2006 (1975kg/ha).

The content in chlorophyll „a” has a more reduced value than the other studied species, with the exception of *Callendula officinalis*. Its value has diminished during vegetation with almost 6% as compared to values recorded in May.

Chlorophyll „b” has the highest values from all the studied species, diminishing with only 7 % during vegetation.

The chromatograms carried out in UV 254 nm reveal no strips noticeable in front of the sinensetin, since the flavonoid specific to this plant is the hesperidin with a metoxilate flavonoidical structure, similar to that of sinensetin.

Hesperidin, alongside flavones and flavonals, is part of vitamin P composition. The basic role of the flavonoids is that of enhancing the capillaries' resistance, of adjusting the absorption at that particular level, contributing, together with vitamin C, to preserving the health of the conjunctive tissue.

The visualization in UV 365 nm, before and after spraying, reveals the yellow-brown strips corresponding to flavonoids, and the blue strips corresponding to polimetoxilated flavonoidical compounds.

Following the spectrophotometrical dosing of the flavonoids as expressed in rutozid, a value of $0,19 \pm 0,06$ % was obtained.

The flavonoids and the vitamins act synergistically. The immune – stimulating and anti-cancer effects of both of them have been confirmed. These substances hydrolyze

under the influence of intestine bacterial environment, transforming into biologically active aglicons.

Mentha piperita

This culture started by planting the stolons in March 2006, when the air temperature varied between 5,8-14,4 °C, and the atmospheric humidity was 65%.

From the mint leaves was determined the quantity of essential oil. According to the monograph of essential plant oil determination - the European Pharmacopoeia, 6th edition, EDQM, 2008, the quality condition is 12 ml/ essential oil/kg of dry plant.

The obtained essential oil has a light yellow color and a strong scent of menthol. In sample a (leaves harvested in August, day, 14.00 p.m.), the concentration of essential oil was 0, 71 %. Sample b, containing leaves harvested in October, at 20.00 p.m., indicate a concentration of 0, 37%. This result can be explained by the fact that, at night the substances produced during photosynthesis in daytime, migrate from the leaves towards other organs of the plant.

The chromatograms identifying the terpenic constituents in the essential oil on slim layers show the separation of terpenic compounds specific to the essential oil, identifying more precisely the menthol, in the leaves' extract as well as in the separate essential oil. Based on the comparison of R_f values and the comparison of the strips' aspect and those of the norm, one notices that the essential oil contains less constituents as the dichloromethanic leaves extract, since this solvent derives other classes of active compounds as well.

The menthol stimulates secretion and the gall-bladder as well as liver functions. It is choleric and colagog, encouraging the production of gladder and positively influencing the fluidity, at the same time helping the bladder relieve.

The leaves of *Mentha piperita* are registered in the European Pharmacopoeia monograph, 6th edition, EDQM, 2008.

In 2008, the separate as well as the cumulated effect of treatment with growth stimulating and retarding substances has been monitored. The plants were sprayed during the vegetative growth.

The analysis was carried out on the production of fresh leaves, herba, fresh mass constituents and herba etheric oil production.

The largest production was recorded in the case of the variant treated with giberillic acid. Higher values than in the case of the control sample were also recorded at the variants treated with AIA and 6-Benzyladenine. Cycocel and Ethrel have deterred the vegetative growth, thus inducing smaller values in the case of the control sample. Herba leaves production is also superior to that of the control samples of variants treated with giberillic acid, AIA and 6-Benzyladenine, while Cycocel and Ethrel have deterred the vegetative growth.

At DL of 0,1%, production has increased with almost 50%.

As far as the accumulation of etheric oil in mint plants is concerned, all the substances have positive effects. A.I.A stimulates the most the synthesis of these oils, and giberillic acid and Ethrel the less.