COMPARATIVE EVALUATION OF PRODUCTION POTENTIAL OF STANDARDIZED RAW VEGETAL MATERIAL FOR TRITERPENIC ACIDS CONTENT USING CONVENTIONAL AND ECOLOGICAL TECHNOLOGIES OF CULTIVATION APPLIED TO *OCIMUM BASILICUM* AND *SATUREJA HORTENSIS* SPECIES

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Abstract

The complex biological activity of triterpenic acids, exerted on vital organs in different pathological conditions, imposed finding the solutions for their applicability in pharma industry. These solutions are conditioned by the use of industrial technologies working with standardized raw vegetal material in triterpenic acids content, which are available in industrial quantities and obtained through reproducible cultivation technologies applied on large areas.
The assessment of production potential for raw vegetal material with high triterpenic acids content of the mentioned medicinal plants was studied on extended areal, which covered the south, east and central parts of Romania, taking into account the results obtained by Biotehnos, SCDA Secuieni and USAMV Cluj in their own experimental fields.
The best results obtained at *Satureja hortensis* species were those when the plants were cropped in phenophase III and the drying was done in natural shadow conditions. In that phase of harvesting the plants were at growing and developing phenophase when over 50 % from total number of principal inflorescence had mature fruits. Natural shadow drying induced the increase in triterpenic acids content for both cultivation technologies, excepting the harvest made in phenophase I. The triterpenic acids content is higher in herba obtained in ecological cultivation system for the phenophases I, II and IV. The herba production in ecological system was higher for the harvest phases I and II than the conventional cultivation system, which in change was superior in herba production in phenophases III and IV.
The triterpenic acids content from herba of *Ocimum basilicum*, dried at shadow, had a tendency of increase starting with the harvest phase III. In the ecological cultivation system the triterpenic acids content from herba was higher than in the herba obtained with conventional technology, for all harvest phases, excepting the third one. The shadow drying of plants determined the increase of triterpenic acids content in both cultivation systems. The highest level of triterpenic acids production from *Ocimum basilicum* herba was achieved in the last phase of harvest (V).
Taking into account the presented results, thus was proved the utility of identified technological models for the production of standardized vegetal raw material for triterpenic acids content from the studied species of medicinal plants.

Key words: standardized, triterpenic, raw material, production

Products' quality continuous enhancement represents the major goal of standardization activity in the market economy. Thus, the international market segment for phytopharmaceutical products is in continuos development atracting numerous pharma companies including multinationals.

Taking into account this ascending economical trend, numerous international organizations as World Health Organization (WHO), European Council and others, at local level, emmitted a series of reglementations regarding cultivation, harvesting and processing of raw vegetal materials for production of phytopharmaceuticals, such as **WHO Guidelines on Good Agricultural and Collection practices-GACP- for medicinal plants, Geneva, 2003** and CE directive for medicinal plants (**DIRECTIVE 2004/24/EC**).

According to the new ecological orientation towards quality life improvement, both on global and national level, there was an increasing attention on phytotherapy in the last decade, which determined a diversification and intensification of phytochemical and pharmacological researches towards medicinal plants' valorization in different areas: human and veterinary therapeutics, food and cosmetic industries (Jones, W.B., 1998).

The pharmacological studies towards medicinal' plants valorization in therapeutics have significantly evolved, according to the general progress of cellular and molecular biology and

biochemistry representing the premise of a new therapeutical orientation towards their use as new medicines in major clinical areas with large social incidence Newman (Cragg, D.J. et al., 2000, Istudor ,V., 2001).

A group of such vegetal substances is represented by triterpenic acids with α or β amyrinical structure, presented in free or glycosydated state in many medicinal plants, among there are the two ones taken into present study: *Ocimum basilicum* (basil) - fam. *Lamiaceae* and *Satureja hortensis* (thyme) - fam. *Lamiaceae* (Liu, J., 1995; Van Baren, C. et al., 2006, Bina, S.S.et al., 2007.

The applicability of these compounds in pharmaceutical and cosmetic industry is conditioned by their assurances with triterpenic acids' standardized raw vegetal material, in industrial amounts, using reproducible cultivation technologies, as this paper will present.

MATERIAL AND METHOD

The two mentioned plant species were cultivated in conventional and ecological systems in three locations: Biotehnos (Ungureni, Dambovita County), Cluj University of Agricultural Sciences and Veterinary Medicine (Cluj Napoca, Cluj County) and Secuieni Agricultural Research & Development Station (Secuieni, Neamt County). In these geographical areas the plants were harvested in different phases of growing and developing, the researches being done between 2008-2009. The plants were sun, shadow or artificial dried (Plugaru, V., 2000, Verzea, Maria et. al., 2002, Varban, D.I. et. al., 2005).

The raw vegetal material of Ocimum basilicum specie (HERBA BASILICI) is formed of young branches and stalks, partially covered with leaves and having or not terminal inflorescences.

The phenological phases of harvesting were established with regard to the development dynamics of central and principal inflorescences:

- f_1 – when buds appear on verticiles disposed at plants' third basal;
- f_2 – open flowers on verticiles disposed at plants' third basal;
- f_3 – immature fruits disposed at plants' third basal;
- f_4 – mature fruits disposed at plants' third basal;
- f_5 – central inflorescence has mature fruits on most of the verticiles.

The raw vegetal material of *Satureja hortensis* specie (HERBA SATUREJAE) is represented by young branches with leaves having inflorescences with small white-pink colored flowers.

The phenological phases of harvesting were established with regard to the development dynamics of the major branch inflorescences:

- f_1 – buds appearance at the first 2-3 verticiles on over 50% from the principal inflorescences;
- f_2 – at flower opening from the verticiles situated in the first half of the principal inflorescences length;
- f_3 – at the first fruits maturation on over 50% from the principal inflorescences;
- f_4 – at mature fruits formation on most of the principal inflorescences.

There were kept probes for chemical analysis and there were evaluated the green and dried productions for each phenophase.

The experimental variants from the conventional agricultural system were treated with liquid mineral fertilizers. The weed control was done using herbicides, manual works (hand weeding and hoeing) and mechanical hoeing. For ecological experimental variants the organic fertilizers application was done at prelimination and the maintenance of cultures was assured with manual works and mechanical hoeing.

The drying was done naturally (at sun or shadow conditions) and artificially using 1-2 shelf temperatures.

The raw vegetal material production was expressed in q/ha and the evaluations were done both bon fresh and dried matters. Thus there was established the optimal harvesting phenophase when the raw vegetal quantity and the triterpenic acids content had both maximal values. Also the drying process was done in different ways to see how that affected the triterpenic acids' content.

The potential of vegetal production and respectively triterpenic acids' content for these plant species was done at different phases of harvesting and for both, conventional and ecological crop systems. The results are presented as mean of the values obtained in the three experimental locations, in order to obtain an overall production potential for both medicinal plant species taken into study.

RESULTS AND DISCUSSIONS

Satureja hortensis L. – thyme

There were done four production evaluations which corresponds to distinct phenophases in order to determine the optimal period of harvesting.

Harvest period I

This harvest period corresponds to the phenophase when the buds appear on the first 2-3 verticiles at over 50 % from the total number of principal inflorescences, the results being presented in *table 1*.

The plant humidity at this harvest period was of about 70% and the dried herb productions obtained were of 32 q/ha in the conventional system, and of 35 q/ha in the ecological one.

Table 1

Thyme production potential evaluation at harvest period I.

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		106.61	116.61
Dried herba production - q / ha -		32.02	35.01
Triterpenic acids' content g at 100 g f.w..	Sun drying	1.46	1.19
	Shadow drying	0.96	1.03
Triterpenic acids' production kg/ha	Sun drying	46.59	41.93
	Shadow drying	30.62	36.19

The triterpenic acids content was bigger at the sun dried plants in both crop systems. The triterpenic acids productions was of 46.59 kg in the conventional system and of 41.93 kg/ha in the ecological one, when the sun drying was used.

Harvest period II

The thyme plants are characterized by the flowers opening on the verticiles situated in the first half of the principal inflorescences. The data regarding vegetal production and triterpenic acids' content are presented in *table 2*.

Table 2

Thyme production potential evaluation at harvest period II

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		143.91	156.01
Dried herba production - q / ha -		48.92	53.01
Triterpenic acids' content g at 100 g f.w..	Sun drying	1.01	1.49
	Shadow drying	1.27	1.54
Triterpenic acids' production kg/ha	Sun drying	49.49	78.86
	Shadow drying	61.96	81.67

The fresh herba production in ecological system was with 12.1 q/ha bigger than the one from conventional system taking into account the fact that the plant humidity was of 66 %.

The triterpenic acids' content was bigger when shadow drying was used from 1.27 g at 100 g fresh weight (f.w.) instead of 1.01 g at 100 g f.w., when sun drying was applied on conventional culture and of 1.54 g at 100 g f.w.., compared to 1.49 g at 100 g f.w. for sun dried ecological plants.

The triterpenic acids' productions were bigger when shadow drying was applied for both cultivation technologies, of 61.96 kg/ ha in conventional system and of 81.67 kg/ha in ecological one, compared to 49.49 kg/ha and 78.86 kg/ha with sun drying.

Harvest period III

This phenological phase was characterized by mature fruits formation at over 50% from principla inflorescences and with plant humidity at harvesting of 53%.

The estimation of herba and triterpenic acids' production and their content for both crop systems are presented in *table 3*.

Table 3

Thyme production potential evaluation at harvest period III

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		119.21	107.32
Dried herba production - q / ha -		56.01	50.41
Triterpenic acids' content g at 100 g f.w..	Sun drying	0.42	0.47
	Shadow drying	4.06	3.76
Triterpenic acids' production kg/ha	Sun drying	23.46	23.53
	Shadow drying	227.47	189.45

This phenophase is distinguished by the big triterpenic acids production in both cultivation systems when the vegetal biomass was shadow dried.

The dried herba production was between 56 q/ha for conventional crop and of 50.41 q/ha for ecological one.

In the conventional culture system the triterpenic acids' content was of 4.06 g at 100 g f.w., with a crop production of 227.47 kg/ha, compared with 3.76 g triterpenic acids at 100 g f.w. and 189.45 kg/ha crop production for the ecological one.

Harvest period IV

The specificity of this period is mature fruits formation on the majority of plants 'inflorescences. In these conditions, when the plants humidity was of 45% at harvest, the fresh herba productions, in the both crop systems, registered the smallest values, presented in *table 4*.

Table 4

Thyme production potential evaluation at harvest period IV

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		95.11	81.42
Dried herba production - q / ha		52.31	44.82
Triterpenic acids' content g at 100 g f.w..	Sun drying	0.44	0.37
	Shadow drying	3.61	3.99
Triterpenic acids' production kg/ha	Sun drying	22.51	16.66
	Shadow drying	88.69	178.79

The dried herba production in the conventional system was of 52.31 q/ha, compared to just 44.81 q/ha in the ecological system, and the triterpenic acids' content was of 3.99 g at 100 g f.w. in the ecological one and of 3.61 g/ 100 g f.w. in the conventional system, both for shadow drying conditions. The triterpenic acids' productions obtained in this period are lower than in the third one, but superior to those from first and second periods, thus being of 178.79 kg /ha in the ecological system and of 188.69 in the conventional one.

***Ocimum basilicum L* - basil**

There were established 5 harvest periods correlated with the plants' dynamic growth and development, in order to evaluate herba and triterpenic acids productions at basil.

Harvest period I

The basil plants, at this time of harvest, have buds at the verticiles from the third basal of the central and principal inflorescences. In Table 5 there are presented the partial results regarding the production level obtained in this phenophase, when the plant humidity at harvesting was about 81%.

The dry herba production in the ecological crop system was of 25.5 q/ha, instead of 23 q/ha in the conventional one. The triterpenic acids' content from ecological herba was of 1.23 g at 100 g f.w., thus obtaining a production of 32.46 kg/ha, and of 1.24 g at 100 g f.w., for the herba obtained in the conventional crop system, which had a production of 28.47 kg/ha (*table 5*).

Table 5

Basil production potential evaluation at harvest period I

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		121.01	134.02
Dried herba production - q / ha -		23.02	25.51
Triterpenic acids' content g at 100 g f.w..	Sun drying	0.63	0.43
	Shadow drying	1.24	1.27
Triterpenic acids' productio kg/ha	Sun drying	14.47	10.86
	Shadow drying	28.47	32.46

Harvest period II

The harvest phenophase at this period corresponds to the flower opening at the verticiles from the third basal of the central and principal inflorescences, and the plant humidity was about 77%.

In the ecological crop system the herba and triterpenic acids' productions for shadow drying conditions were higher than the ones obtained in the conventional system of cultivation.

Thus, the higher triterpenic acids' content in the ecological shadow dried herba, determined a triterpenic acids' production of 61.91 kg/ha instead of 35.32 kg/ha in the conventional one (*table 6*).

Table 6

Basil production potential evaluation at harvest period II

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		147.01	155.02
Dried herba production - q / ha -		33.81	35.62
Triterpenic acids' content g at 100 g f.w..	Sun drying	1.01	0.71
	Shadow drying	1.05	1.74
Triterpenic acids' production kg/ha	Sun drying	33.83	25.13
	Shadow drying	35.32	61.91

Harvest period III

The third harvest period was established in the phenophase when basil plants had immature fruits at the verticiles from the third basal of the central and principal inflorescences, with a plant humidity of 73%.

The herba productions, triterpenic acids' content and their production values are presented in *table 7*.

The dried herba production in this harvest period is also bigger in the ecological crop system (50.7 q/ha), than the one obtained with the conventional system (47.6 q/ha).

The basil herba obtained with the conventional crop system had a bigger triterpenic acids' content which determined a production of 80.44 kg/ha, instead of 69.46 kg/ha in the ecological system.

Harvest period IV

The characteristics of this harvest period are the formation of mature fruits at the verticiles from the third basal of the central and principal inflorescences and the plants' humidity is 70%. The triterpenic acids' content and the productions obtained with the two cultivation technologies are presented in *table 8*.

The data from the table show that the herba production obtained with the conventional cultivation system (sun or shadow dried) is superior to the one obtained in the ecological system, being also bigger for both crop technologies comparative with the productions obtained in the precedent harvest periods.

The triterpenic acids' content in the ecological herba was 2.89 g at 100 g f.w., instead of 2.19 g at 100 g f.w., in the conventional cultivated herba. Thus the production of triterpenic acids was of 173.98 kg/ha in the ecological system instead of 144.47 kg/ha in the conventional one.

Harvest period V

The plants harvesting in this period was made during the formation of mature fruits at most of the verticiles from central and principal inflorescences and with a plant humidity of 65%.

The productions obtained for fresh herba were smaller in both cultivation systems, comparative with the precedent harvest period, as presented in *table 9*.

The triterpenic acids' content in the shadow dried herba has the biggest values for both cultivation systems from all the previous harvest periods. Thus, the content at basil ecological crop was of 3.16 at 100 g f.w. and of 2.97 g at 100 g f.w. for the conventional one.

In this harvest period there were obtained the biggest triterpenic acids' productions for both cultivation technologies, their values being relatively close of 216.03 kg/ ha with ecological system and 218.61 kg/ha with the conventional one.

Table 7

Basil production potential evaluation at harvest period III

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		176.41	188.01
Dried herba production - q / ha -		47.61	50.72
Triterpenic acids' content g at 100 g f.w..	Sun drying	1.56	0.94
	Shadow drying	1.69	1.37
Triterpenic acids' production kg/ha	Sun drying	74.26	47.66
	Shadow drying	80.44	69.46

Table 8

Basil production potential evaluation at harvest period IV

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		220.01	200.02
Dried herba production - q / ha -		66.01	60.11
Triterpenic acids' content g at 100 g f.w..	Sun drying	0.43	0.69
	Shadow drying	2.19	2.89
Triterpenic acids' production kg/ha	Sun drying	28.58	41.35
	Shadow drying	144.47	173.98

Table 9

Basil production potential evaluation at harvest period V

Production, content		Cultivation system	
		Conventional	Ecological
Fresh herba production - q / ha -		210.01	195.02
Dried herba production - q / ha -		73.61	68.31
Triterpenic acids' content g at 100 g f.w..	Sun drying	0.74	0.89
	Shadow drying	2.97	3.16
Triterpenic acids' production kg/ha	Sun drying	54.39	61.19
	Shadow drying	218.6	216.03

CONCLUSIONS

The experimental researches done on *Satureja hortensis* specie, regarding the production potential of triterpenic acids, showed that the best best results are obtained when plants were harvested in the third phenophase and the dryinh was done in shadow natural conditions. In this harvest period the plant are in the phenophase of growing and developing when over 50 % of total principal inflorescences have formed mature fruits.

The natural shadow drying determined the increase in triterpenic acids' content for both cultivation systems, excepting the variant harvested in the phenophase I.

The triterpenic acids' content was bigger in the herba obtained with the ecological cultivation system for harvest periods I, II and III.

The herba productions with ecological technology were bigger for harvest periods I and II comparative with the conventional crop, which, instead, is superior in the third and fourth harvest periods.

In conclusion, at ***Satureja hortensis* L.**, the biggest production of herba in the ecological system was for the variant harvested in the third phenophase (the formation of immature fruits at verticiles from the third basal), the increase compared with control (the floral buds appearance at verticiles from the third basal) being 1608.33 kg/ha; the experience made with conventional crop technology produced the biggest dry herba production of 3075 kg/ha (compared to the control which had 1758.33 kg/ha). Regarding the triterpenic acids' content, this parameter was higher at dry artificial conventional crop variant harvested in the phenophase 4 (3.98%) and smaller for the ecological crop variant harvested in the phenophase 3.

At ***Ocimum basilicum*** specie the triterpenic acids' content from the basil herba obtained through shadow drying had a continuous increasing tendency starting with harvest period III.

The ecological crop determined a superior triterpenic acids' content in the ecological herba than in the conventional one,for all harvest periods, excepting the third phenophase.

The plants' shadow drying determined the raising of triterpenic acids in both crop systems.

The maximum level of triterpenic acids' production obtained from basil herba was achieved in the last period of harvest (V), reaching the values of 216.03 kg/ ha in ecological system and 218.61 kg/ha in the conventional one.

In conclusion ecological technology applied to basil determined the highest production of dried raw vegetal material (2908.34 kg/ha) obtained at phenophase 3 (the formation of immature fruits at verticiles from the third basal); at variants obtained with the conventional technology, the biggest production was obtained in phenophase 3 with 3083.33 kg/ha compared with control which had 1725 kg/ha. The triterpenic acids' content, obtained by analyzing a part from all basil variants, was the highest (2.73%) at shadow dried variants harvested in the phenophase 4.

Taking into account the fact that on the national and international level the agrobiological researches regarding the plant amelioration have remained after phytochemical studies, and, until now, there were not experimentally obtained any varieties with high triterpenic acids' content which could be used to produce standardized raw vegetal materials for pharma industry, we consider that this type of study is of high interest and topicality.

Our recommendations and research experience could be useful to a beneficiary who wants to make triterpenic acids production at industrial level for conditioning them as drugs, thus being able to grow such plant species as standardized raw vegetal material in these active biocompounds, eliminating, in this manner, the lack of reproducibility and applying a profitable industrial technology.

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