Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 6 (55) No.2 - 2013

ECOLOGICAL VARIABILITY OF L-ASCORBIC ACID IN *PRIMULA TAXA* FROM POSTĂVARU MOUNTAIN

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Abstract: *L-Ascorbic acid (C vitamin) is currently used for many purposes, especially in pharmaceutical industry. There are important plant resources for this substance around the world, in fruits or leaves. For example, in Romania, "**Primulae folium**" (leaves from Primula sp.) are used in ethnopharmacology. The present study aims to identify potential relations between different local climatic factors (such as air temperature and humidity, wind speed) and C vitamin content in "**Primulae folium**". The performed case study, emphasized a contribution of the meteorological variables to the C vitamin content in Primula sp. up to 22-58%, whose maximum values were located around middle of May and in the interval between* 14^{00}-16^{00}.

Key words: *C vitamin content, Primula elatior, Primula veris, meteorological variables.*

1. Introduction

The increased popularity of the L (+) - Ascorbic acid, which is the biochemical identity of C vitamin, is a result of its therapeutic importance. One of its benefits for the human organism is reflected by the its increased capacity of resistance which is generated by the stimulation of the leucocytes phagocytosis [14].

This substance is valued in medical applications (allopath and naturopath procedures), for many reasons such as: oxy-reduction properties of the ascorbic acid complex, active participation to cell respiration as well as the formation of capillary and connective tissue [1].

The most generous C vitamin suppliers are the ligneous plants from forest ecosystems: wild rose, sea buckthorn, walnuts and mountain ash. In the leaves of Romanian *Primula* species, C vitamin is a compound of secondary importance contributing with up to 3% to the overall chemical composition [19], while the chemical architecture is dominated by saponins [8], [20] and triterpenoids [13] having expectorant and secretolytic properties [2], [6], [23].

Applications of "***Primulae folium***" – the pharmacological denomination of the medicinal product resulted from dried leaves of Primula species – are well-known in international ethnopharmacology. The

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leaves are used for multiple purposes such as treating epilepsy and convulsions as well as for sedation purposes [16]. Some *Primula* taxa were tested and proved antibacterial values [15].

Primula veris L. (*P. officinalis* (L.) Hill.) and *Primula elatior* L. (Hill.) are the only taxa from Romanian flora having a pharmacologically confirmed therapeutic value [19]. These species are fully utilized in ethnopharmacology [4], the leaves vitamins content being commonly extracted using infusion [7].

The variable conditions induced by environmental factors, which generate variability in growth and individual development phases, represent sufficient premises for an ample variability regarding the active substances of spontaneous flora. They could also represent the main impediments regarding the dosage of ingredients when dealing with applications in natural beauty and health resources processing. The impact of this variability on final products, justified the effort to identify the local climatic factors which could act as controllers of the content of the active substances. Expression of the relations between the dynamics of local climatic factors and the content of active substances in the leaves may help deciding the best (optimum) moments for harvesting the raw material, with application in allopathic or naturopathic medicine.

2. Objectives

Local and regional climate influence the plants growth as well as the accumulation of different substances. Since the *Primulae folium* has a widespread utilization in the traditional medicine, the objectives of this paper were to mathematically express the relation between the C vitamin content and the main weather factors as well as to identify the optimum moment for leaves harvesting.

3. Material and Methods

3.1. Study Location

The wide spreading of *Primula* taxa around Braşov circumscribes itself to the pedological, hydrological and climatic conditions offered by the natural environment of Postăvaru Mountain - a geomorphological complex which presents almost all the mountain-area specific vegetation storeys. Intra-mountainous depression specific vegetation is also present [17]. These conditions are suitable for edaphic requirements of the mentioned species [11], explaining at the same time the presence of *Primula veris* on skeletal soils and that of *Primula elatior* on groves and meadows with loose soils.

3.2. Study design

The field acquired data was provided by the deployment of a sample plots network along a transect positioned on the north-western slope of Postăvaru Mountain, near Braşov - Romania. The altitude of the transect ranged from 723 m up to the mountain's top and contained three permanent sample plots as well as five control points, located in meadows near the tree line (Table 1).

The sample plots were periodically visited between the 1st of April and 23rd of June 2011. The initial sampling placement has been completed by adding another six control points, distributed on the entire transect, which were visited only once. The sample plots were visited in the hourly interval 10^{00}-18^{00}.

During each visit, leaf samples were harvested. The material belonging to *Primula veris* was harvested only from the second sample plot. Therefore, the main studied species was *Primula elatior*. Instrumental observations concerning some meteorological parameters were also performed during the sampling activity.

They relate to the air temperature, air humidity and wind speed.

Table 1

Geomorphological identification of the experimental sample plots

Indicative of the sampling area	Altitude m	Local topography	Slope aspect
S1	723	Low side slope	NW
S2	805		
S3	727		
P4	1144	Middle side slope	
P5	1255		
P6	1374		
P7	1452	High side slope	
P8	1674		
P9	1778		NE

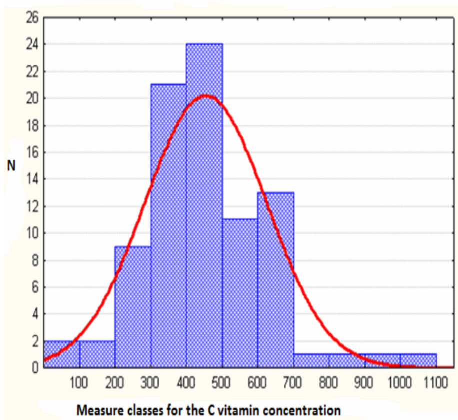
The harvested material was conditioned by laboratory freezing in order to preserve it for the time period between harvesting and determination of the physical and chemical indicators - content in ascorbic acid. The field sampling was planned and organized in order to quantify the weather state influence on the leaf content in the active substance, as well as to determine the best moment for the harvesting of vegetal material. There were adopted the following hourly harvesting intervals: 10^{00}-12^{00}, 12^{00}-14^{00}, 14^{00}-16^{00} and 16^{00}-18^{00}. These harvesting intervals served to the ulterior stratification of laboratory determined indexes. Leaf harvesting was interrupted during rainy days. The weather was assessed by considering the following parameters: relative atmospheric humidity (W_r, %), atmospheric temperature (T, ^{0}C) and wind speed (V_{max}, m/s). Values of parameters were measured at ground level as well as at 30 cm height above ground using a multifunctional device produced by EXTECH Instruments, model 45160.

During field visits, from each permanent sampling area and control point, three healthy and clean leaves belonging to five different plants were harvested – forming five samples (of three leaves each one); these were hermetically packed (in order to preserve the original humidity), labelled and transported to the laboratory.

In order to determine the physical and chemical indexes, smaller parts were extracted from the main samples. Determination of relative humidity took into consideration the use of fresh leaves, while C vitamin content was determined using refrigerated leaves. Laboratory analysis was performed in order to determine also the artificial drying duration as well as the chromatic parameters. In order to estimate the C vitamin content, which was the purpose of this study (Γ_C, mg vitamin/100 g leaves), there was used the iodometric method [9-10] consisting of the extraction in HCl 2%.

4. Results and Discussion

Wide dispersion of the determined values of C vitamin content (Figure 4) emphasized the remarkable dynamics of the biogenesis of substance in *Primula* leaves The central trend, determined by an extreme variability (variation coefficient was greater than 40%) as well as by median value, located the C vitamin content in *Primula veris* at the same level as pepper or black currants. Particularly interesting are the values presented by the upper variation limit which was comparable with C vitamin concentration from the sea buckthorn or even from the wild rose in certain stages of their development. The experimental distribution of C vitamin content in *Primulae folium*, which was asymmetric related to the normal distribution (Figure 1), led the mathematical analysis in the nonparametric statistics domain. The significant statistical differences (t=11.85***) between determinations claimed the identification of variation causes. In order to identify the probable sources of variation, there was used the nonparametric test of ranks [12] [24].



Variable	Descriptive Statistics								
	Valid N	Mean	Confidence -95.000%	Confidence 95.000	Median	Minimum	Maximum	Std.Dev.	Coef.Var.
C vitamin, mg/100 g	120	665.94	554.65	777.22	470.22	52.84	3156.91	615.66	92.45

Fig. 1. *Experimental distribution and statistics of C vitamin content for the analyzed material*

In what concerns the C vitamin content, the harvesting moment of samples represents one of the primary variation factors (Table 2).

Table 2

Statistic significance of influence of some independent variables on the C vitamin content using Kruskal-Wallis test

Variable	**DF**	***H***	***p* [%]**	**Sig.**
Harvesting date	8	62.09	<0.001	***
Harvesting hour	3	29.91	<0.001	***

The influence of weather parameters, having a continuous variation domain on the active substance from the harvested leaves, was examined by considering the value and significance of nonparametric simple correlation coefficients (Table 3). For the vitamin content, some variables acted in a stimulative direction (air temperature - T, wind speed – V_{max}) and other in a depreciative direction (air humidity – W_r). The most stable influence on the static vitamin content was represented by the air temperature (Table 3).

Table 3

The mathematical relation between the C vitamin content and the independent variables

Pairs of variables	***N***	***R***	***t*(*N*-2)**	***p* [%]**
T & Γ_C	83	0.764	10.66 ***	< 0.001
W_r at ground & Γ_C	83	-0.543	-5.82 ***	< 0.001
W_r at 30 cm above ground & Γ_C	77	-0.514	5.19 ***	< 0.001
V_{max} & Γ_C	28	0.475	2.75 *	1.06
Altitude & Γ_C	86	0.457	4.71 ***	< 0.001

Direction and intensity of these influences, provided by the measure and sign of correlation coefficients, will be analysed next, in order to better understand the conditions which influence the intra-annual dynamics of active substances. The variation of C vitamin concentration followed closely (Figure 2) the air temperature dynamics, emphasizing the link between the two variables offered by Spearman correlation coefficient.

An exception was observed at the middle of April when the proportionality direction was locally modified, without a valid explanation among the analysed factors. The influence of the temperature and humidity seemed to be independent. The C vitamin regress related to atmospheric humidity was not absolute, as proved by the 15th of April and final observations values (Figure 3).

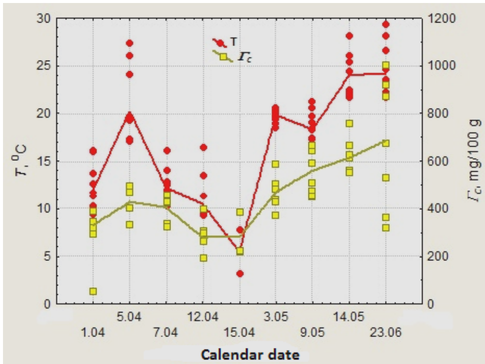


Fig. 2. *C vitamin dynamics related to the air temperature*

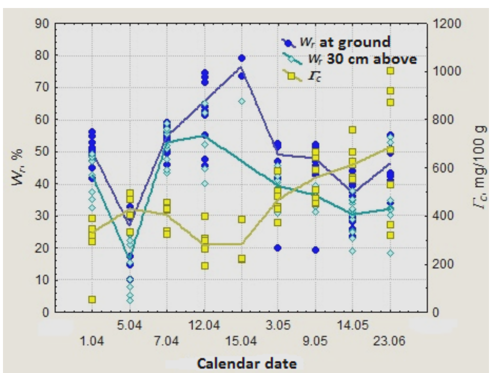


Fig. 3. *C vitamin dynamics related to the air humidity*

It was remarkable the continuous increment of C vitamin content after the first two decades of April depression (concomitant with the snow layer reappearance).

If in the case of air temperature and humidity the inverse proportionality was statistically visible (*R* = -0.75..-0.69), the punctual wind speed had no correspondence with the above mentioned (*R* = -0.01...0.10). In the absence of these interactions, the individual relation of C vitamin content in rapport with the wind speed (Figure 4) became the physiological consequence of the action of water loss under cellular juice concentration – the increase of air masses circulation (up to some limits) stimulates the water loss and thickens the cellular juice solution.

Thus, the reaction of C vitamin content at high amplitudes of circulation of air masses was more tempered in comparison with the modification of the same dosages in the thermal or pluvial regime.

A similar explanation can sustain the C vitamin concentration in rapport with the altitude (Figure 5) as it represents an ecological determinant for every manifestation of vegetation [5].

The relation between the altitude and vitamin content (statistically proved n *R*=0.457***) masked the influence of main factors which are interacting at the altitude's cover. Two probable explanations may sustain the C vitamin increment related to the altitude – thermal inversions – which occurred during the moments of determination and the modification of solar light spectrum.

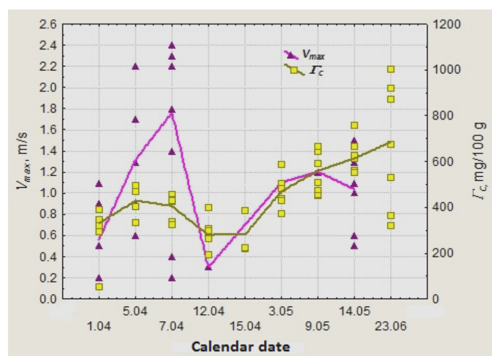


Fig. 4. *C vitamin dynamics related to the wind speed*

The location of ascorbic acid synthesis in chloroplasts, as well as its participation to the photosynthetic transformations chain [14] subdued the active principle content to all the laws of the altitudinal ascension, mediated by the latter's influence – mainly by spectral composition and intensity modification of solar radiation [18]. The increment of long wave visible radiation with altitude, especially ultraviolet radiation, corroborated with *b* chlorophyll sensitivity for this wave length [21] constituted, in our opinion, the safest ground for explaining the C vitamin content behaviour related to the elevation.

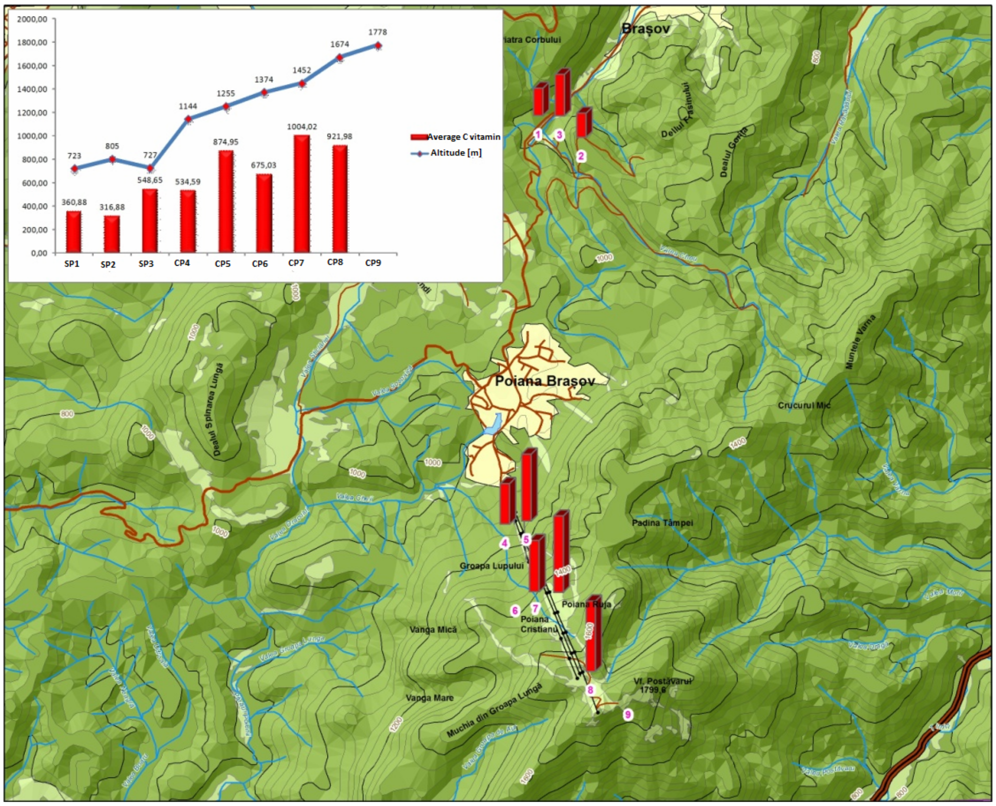


Fig. 5. *C vitamin variation related to the altitude (23rd June 2011)*

A similar causality defined the daily variation of cellular juice concentration in C vitamin. The maximum observed value, recorded in the 14^{00}-16^{00} hourly interval (Figure 6), reflected both, the daily dynamics of photosynthesis process (which encounters, in normal water supply conditions, a maximum value during noon [21]) and cellular turgescence imposed by the water loss dynamics related to environmental humidity (the relative air humidity presented, in the determined samplings, a depression during the 12^{00}-14^{00} hourly interval, which was more accentuated at 30 cm above ground level).

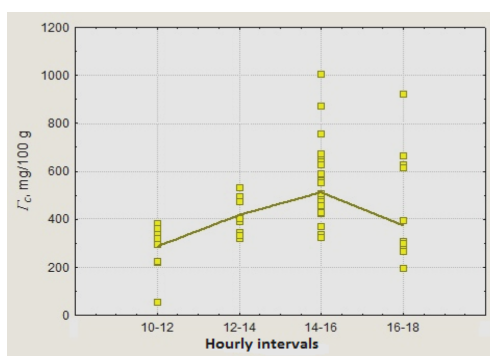


Fig. 6. *Diurnal variation in C vitamin content on the analysed material*

By comparison, the seasonal variation of active principle concentration has to be followed in several consecutive vegetation (life) cycles in order to forecast certain laws to predict the producers' behaviour.

The determinations done during 2011 emphasized the profound impact of the weather (especially its cooling) on the C vitamin presence in leaves. If its dynamics is corroborated with the variation in time of meteorological parameters and the relations between them, the best moment for harvesting the leaves was located in the middle of May.

5. Conclusions

The research presented in this paper followed the identification of potential influence factors regarding the L-ascorbic acid content in *Primula* taxons (*Primula veris* and *Primula elatior*) leaves, and, in relation to their dynamics, the identification of the optimum moment for leaves harvesting and utilization of their therapeutically and alimentary potential. The sampling areas were deployed on the almost entire relief energy of the north-western slope of Postăvaru Mountain.

The ascorbic acid content (independent from an hourly interval to another, from a day to another, from a surface to another and from an altitude step to another, but homogeneous inside these variation factors), varied on the following coordinates:

- The meteorological variables constituted significant influencing factors (***) regarding the C vitamin content values in *Primula* species leaves: in the 2011 season, the air temperature and circulation stimulated ascorbic acid synthesis and in the mean time the atmospheric humidity acted as an inhibitor;
- The contribution to total variation (R^2) of biochemical index (by considering the decreasing importance order) was: 58% for temperature, 26-29.5% for atmospheric humidity and 22.5% for wind;
- The spatial distribution of vitamin content reflected a gradual variation of climatic parameters being responsible of photosynthetic activity rhythms and modifications of vegetation conditions related to the altitude (which explains 21% from C vitamin content variation in the geographical investigation space);
- The maximum accumulation of C vitamin in the vegetal material occurred in the absence of an unfavourable weather intervention (during which the harvesting is not recommended anyway), during the 14^{00}-16^{00} hourly interval, in the middle of May.

The observed averages regarding the vitamin concentrations placed the analysed material on the fourth (honourable) position in the vegetal top of C vitamin producers [3].

Acknowledgements

We would like to thank Eng. Husar Mihai for participating to the field sampling, data collection and centralisation

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