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THE DYNAMICS OF ACCUMULATION OF RUSCOGENIN IN THE ROOTS AND THE RHIZOMES OF *RUSCUS ACULEATUS L.*

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SUMMARY

Certain pharmaceutical preparations are being manufactured based upon ruscogenin, which have wide applications in phlebologic symptoms. An important source of obtaining ruscogenin is from the sub-terranean parts of *Ruscus aculeatus L.* The object of the presented investigations is to follow the phenophasic dynamics of the ruscogenin contents by densitometric method. It has been found that the content of ruscogenin in the roots varies between 0.026 and 0.060 %. The maximum quantity is obtained during the period of full bloom, whereas the minimum is observed during the period of the development of propagatory buds. The sapogenin content varies from 0,05 to 0,07 % in the rhizomes. The maximum quantity is generated at the time of full bloom whereas the minimum is observed at the time of the intensive growth of the new stems. These results exhibit that the most opportune period for the collection of the raw material is during the months May and December for the roots and during August and December for the rhizomes when the content of ruscogenin is the highest.

KEY WORDS

Ruscus aculeatus, steroidal sapogenins, ruscogenin, phenological phases

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INTRODUCTION

Certain pharmaceutical preparations based upon ruscogenin are being manufactured which have wide applications in phlebologic symptoms. An important source of obtaining ruscogenin is from the subterranean parts of *Ruscus aculeatus L.* The object of the presented study is to follow the phenophasic dynamics of the ruscogenin contents by densitometric method.

Literature survey shows an absence of data on this kind of investigation, apart from the studies of the dynamics of the steroidal saponins in *Ruscus ponticus Woronov ex Grossh.* performed only for the calendar months /1/.

PLANT MATERIAL AND METHODS

A wildly grown population of *Ruscus aculeatus*, located in the northern incline of the Belasitza mountain near the village Petrich in SW Bulgaria was studied. The Belasitza mountain relates to the group of high mountains (2000 - 2376 m. alt.) and is non-glaciated. It is situated in the continental mediterranean climatic region; the south-bulgarian climatic sub-region, which is expressed for Bulgaria. Phenological observations and sample collection for analysis was conducted in three experimental areas. They are situated in steep inclines (20p - 30p) and height 600 - 700 above sea level. The rocky base is silicate, the soil conditions are brown forest and comparatively well humidified. The average annual temperature is 8.5 - 12.5 °C. The forest communities are formed by *Castanea sativa Mill., Tilia tomentosa Moench., Platanus orientalis L., Fraxinus ornus L.* Among the scrubs are found *Crataegus monogyna Jacq., Cornus mass L., Colutea arborescens L.*, besides the climber creepers *Hedera helix L.*,

Clematis vitalba L., Tamus communis L. Among the herbs are found *Scilla bifolia L., Lamiastrum galeobdolon (L.) Ehrend. et Polatschek, Lapsana communis L., Cyclamen hederifolium Ait., Clinopodium vulgare L., Scutellaria columnae All., Erytronium dens-canis L. etc.* The registration of the phenological phases is carried out according to the method of Beideman, / 2 / and Golubeva, / 3 /.

The quantitative determination of ruscogenin in the investigated material was performed by the densitometric method Nikolov et al. /4/. Linear filter single ray densitometer CAMAG T Scanner, model 111 was used.

EXPERIMENTAL

The experimental areas were visited every month for the evaluation of the phenological statutes of *R. aculeatus*. At the same time subterranean parts were collected for phytochemical analysis. They were divided into roots and rhizomes, and dried under natural conditions.

Densitometric investigation of ruscogenin content was conducted using chromatographic plates Kieselgel G Merck (20 cm X 20 cm). The plates were spotted with the following solutions :

Solution 1 10.0 g of each of the air-dried and pulverised material was hydrolysed with 150 ml 5% H_2SO_4, on water bath under reflux for 3 hours. After filtration the plant material was neutralised by washing with water, air dried and extracted three times each with 100 ml petroleum ether. After filtration the extract thus obtained was dried and residues were dissolved in 10 ml CH_3OH. 0.020 ml each of the methanolic solutions was applied at the starting point 1 with the automatic device for spotting of the probes on Camag Linomatt III.

All the samples of roots and rhizomes of *R. aculeatus* were processed by the same method. *Solution 2* - 0.002 g ruscogenin was dissolved in 2 ml CH_3OH. 3.0, 5.0 and 7.0 µl each of this solution were applied on the starting points 2, 3 and 4.

The system cyclohexane : ethylacetate (1 : 1) was used. The developed plates were air dried, sprayed with a solution of p-dimethylaminobenzaldehide and warmed at 110°C for 10 min. The reddish - brown spots of ruscogenin thus developed were examined densitometrically /4/.

The percentage content of ruscogenin was calculated by the formula :

$$\% \text{ ruscogenin} = \frac{a \cdot c \cdot e}{b \cdot d \cdot f \cdot g} 100,$$

where : a is the height of the peaks of the samples in mm,

b is the height of peaks of the std. ruscogenin sample in mm,

c is the quantity of ruscogenin in grams,

d is the volume of the ruscogenin solution in ml,

e is the applied quantity of ruscogenin solution in ml,

f is the quantity of the samples in grams,

g is the applied quantity of sample solutions in ml.

RESULTS AND DISCUSSION

From the phenological observations carried out on *Ruscus aculeatus* Table 1 eleven phenological phases have been registered. These results correspond to the literature data indicated by Golubeva, for *Ruscus ponticus* / 3 /. Interesting is the fact as observed by our studies, that the late autumn - winter - early spring flowering amidst the mediterranean

climatic conditions characteristic for *Ruscus aculeatus* Rameau et all, /5/ has been established also in the submediterranean climatic conditions which exist in the mountain Belasitza.

The Table 1 shows the results of the quantitative determination of ruscogenin in the roots and rhizomes during the different phenological phases. The content of ruscogenin in the roots varies from 0.026 - 0.061%, and in the rhizomes from 0.046 - 0.071%.

In the roots the maximum concentration of ruscogenin is found in the phases of massive flowering and the appearance of new shoots, 0.061% and 0.055% respectively.

In the rhizomes, the maximum concentration of ruscogenin has been established in the phases of massive flowering (0.071%), and the end of growth of the new shoots (0.068%).

From the obtained results we can soundly recommend that the most suitable period for the collection of *Rhizoma cum Radicibus Rusci.* with the highest concentration of ruscogenin, are the months December and August. These results correspond to the data of Korkashvili and Pcheidze, for the dynamics of the accumulation of steroidal saponins in the roots and rhizomes of *Ruscus ponticus* /1/. However, these authors have not mentioned phenological data in the seasonal development of the species, and rather only for the calendar months.

The present study shows that the biogenesis of ruscogenin in the subterranean parts of *Ruscus aculeatus* is characterised by phenophasical and seasonal rhythm.

TABLE 1. PERCENTAGE CONCENTRATION OF RUSCOGENIN IN THE ROOTS AND RHIZOMES OF *Ruscus aculeatus* L. (n = 3)

Period of collection	Phenological phases	Roots	Rhizomes
05.08.1991	End of the growth of the new shoots	0,029	0,068
05.09.1991	Start of the development of the flower buds	0,033	0,056
19.10.1991	Beginning of flowering	0,030	0,062
25.12.1991	Massive flowering	0,061	0,071
16.02.1992	Start of the development of the vegetative buds	0,026	0,064
14.03.1992	Intensive growth of the vegetative buds	0,038	0,062
04.04.1992	Intensive growth of the vegetative buds + ripening of fruits	0,048	0,062
02.05.1992	Appepearance of new shoots + end of flowering	0,055	0,057
16.06.1992	Intensive growth of the new shoots	0,029	0,046

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