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Variation in Ruscogenin Contents in *Ruscus aculeatus* L. Growing Wild in Southern Turkey

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The ruscogenin contents of the underground and aerial parts of *Ruscus aculeatus* (Liliaceae) collected from 6 different locations in southern Turkey were investigated by HPLC. The highest ruscogenin content (0.12 %) was recorded from the underground parts of *R. aculeatus* collected from Incebel/ Osmaniye area of Turkey. Ruscogenin contents in aerial and underground parts of *R. aculeatus* varied the contents did not vary greatly in aerial parts.

Key Words: *Ruscus aculeatus* L., Ecological variations, Ruscogenin.

INTRODUCTION

Ruscus aculeatus L. belongs to the family Liliaceae and is native to Mediterranean Europe and Africa[1,2]. Alcoholic extracts of its rhizome have been used for the treatment of some veinous ailments for decades[3] and for hemorrhoids and capillary fragility[4,5]. Decoction of the plant has been used as diuretic, sudorific, antipyretic and tonic in traditional Turkish Medicine[6].

Previous chemical studies on the ruscus plant have showed the presence of a series of steroidal sapogenins and saponins, sterols and triterpenes in the plant[7]. *Ruscus aculeatus* contains 4-6 % of a mixture of steroidal saponin compounds including *ca.* 0.12 % ruscogenin, neoruscogenin, ruscin and ruscoside[8-11]. However, *R. aculeatus* contains mainly two steroidal sapogenins. Ruscogenin and neoruscogenin are usually isolated altogether

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and their mixture is commonly named "ruscogenins". They are used for treating circulatory diseases of the lower limbs, hemorrhoids and some inflammatory conditions of anorectal mucosa[12]. Ruscogenins are considered to be the active components of the extract of *Ruscus aculeatus* and some commercial drugs. Drugs based on *R. aculeatus* dry extract exhibit good anti-inflammatory, vasotonic, antihemorroidal and antiulcer effect[13]. In Europe, the plant is available in capsules or tablets containing *ca.* 300 mg each of a dried extract. Ointments and suppositories for the treatment of hemorrhoids are also available[14].

Wild-collection plays still a vital role in the trade of medicinal and aromatic plants in Europe. A number of European medicinal and aromatic plant taxa that are considered to be threatened by collection in Europe owing to their demand in trade have been selected from the national reports in Albania, Hungary, Spain and Turkey for detailed description. Among them, *Ruscus aculeatus* is important species as a source of ruscogenin in the world[15]. *Ruscus* species are becoming vulnerable through over collection in Turkey. Four species of *Ruscus* are naturally found in the wild in different altitudes in North and South Anatolia[1]. One of them is *Ruscus aculeatus* and the collected plant material from flora of the Çukurova region located in Mediterranean area is exported. The highly heterogeneous soil and climatic conditions of the Mediterranean area have resulted in an increased diversity of medicinal plants[16].

The aim of the research was to survey and identify the ecogeographical distribution of *Ruscus aculeatus* from many environments in the Mediterranean region and to find out the variation in their ruscogenin contents.

EXPERIMENTAL

Samples of *R. aculeatus* were collected from six different locations in the Çukurova region in April/May in 2003 during the flowering period. Because the aerial parts of the plants die in winter and new green branches arise in spring; collections were done in April and May. The first collection was done in October 2002 (Table-1). These locations are forested (Taurus Mountains) and on the Mediterranean coast. Plant samples were identified by Dr. Ahmet Ilcim (Biology Department, Kahraman Maras Sütçü Imam University). Voucher specimens are kept at the herbarium, Department of Biology, Kahraman Maras Sütçü Imam University. The collected plants were also planted in the experimental area of the Department of Field Crops, Çukurova University.

Plant materials were separated into leaves and roots, dried at 55°C, ground to homogenous powders and kept at room temperature for further analysis. Dried and powdered underground and aerial parts (20 g for each sample) were treated with 2 N HCl while heating for 8 h in water bath

TABLE-1
Ruscus aculeatus COLLECTING IN ÇUKUROVA REGION, 2002-2003

Site	Date	Altitude (m)	Habitat	Voucher numbers	Morphological Characteristics
Zorkun/ Osmaniye	7.04.2003	750	*Pinus* sp., deforested area, then recovered low trees population, relatively low fertile soils, rocky screes	1348	Small leaf, concave twisted, dark green, rigid, spiny cladodes, stem unbranched, average plant length 26.4 cm
Bahçe/ Adana	29.10.2002	630	Non-forest but recovered from forest currently field crop production area especially maize grazed land.	1349	Small leaf, concave twisted, dark green, rigid, spiny cladodes, stem unbranched few green fruit, average plant length 50.1 cm
İncebel/ Osmaniye	28.04.2003	650	*Quercus* sp., *Pinus* sp., soils covered *Pinus* and other litter, richness in humus, humid soils	1350	Relatively big leaf, smooth, dark green, rigid, spiny cladodes, stem unbranched, a lot of red fruit, thick root and stolon, average plant length 38.9 cm
Düziçi / Osmaniye	29.04.2003	500	*Quercus* sp., *Pinus* sp., Cistus sp., Myrtus sp., *Erica* sp., relatively low fertile soils	1351	Small leaf, concave twisted, dark green, spiny cladodes, stem unbranched, with flowers, average plant length 45.2 cm
Horzum-Kozan/ Adana	1.05.2003	500	*Stachys* sp.,*Pinus* sp., *Olea* sp., *Rhus coria*, *Cappari*s sp., *Astragalu*s sp., humid soils	1352	Relatively big leaf, smooth, dark green, rigid, spiny cladodes, stem unbranched, with flowers average plant length 35.3 cm
Çamliyayla/ Mersin	14.04.2003	1430	*Quercus* sp., *Pinus* sp., soils covered dense *Pinus* litter, humid soils	1353	Relatively big leaf, smooth, dark green, rigid, spiny cladodes, stem unbranched, with flowers, average plant length 36.4 cm

under a reflux condenser. After cooling and filtering the mixtures were washed with a 5 % $NaHCO_3$-solution and H_2O until a neutral reaction is reached. The hydrolyzed products were dried at 65ºC and then extracted with petroleum ether:ethanol (95 : 5) in a soxhelet for 6 h. The solvent was evaporated under vacuum. Each residue was dissolved in HPLC grade methanol and filtered with a 0.45 µ filter (Millipore). The solutions were then made up by dilution with the same solvent. A set of five standard solutions was prepared containing 0.1-0.01 mg/mL of ruscogenins. A 20 µL volume of the solutions were injected into the HPLC system.

The HPLC system includes an Agilent 1100 Quaternary pump and Agilent 1100 Variable wavelength UV detector. Analyses were performed on a Supelcosil LC8 (mean particle size 5 µm) stainless steel column (250 × 4.4 mm I.D). The mobile phase was acetonitrile:water (6:4), flow rate 1 mL/min. Chromatograms were recorded and peak area measured with Chemstation Rev.A09.03 software of the system. The UV detector was set at 200 nm.

The amount of ruscogenin in the sample was calculated from comparisons of peak areas with the calibration graph (Fig. 1) constructed from the standard solutions. Each analysis was repeated three times. Results (on dry weight basis) are given in Table-2. Ruscogenin contents of the aerial and undergrounds parts of *R. aculeatus* growing in southern Turkey were determined separately by HPLC. External standard method was used for quantitative determination.

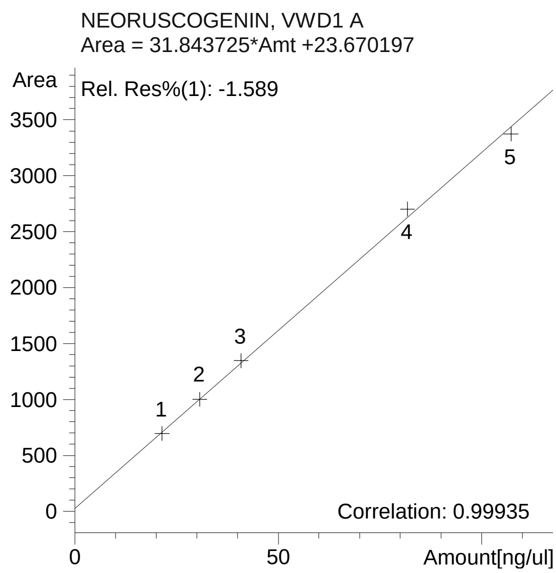

Fig. 1. Calibration graph for neoruscogenin

TABLE-2
RUSCOGENIN CONTENT OF *R. aculeatus* SAMPLES COLLECTED FROM DIFFERENT LOCATIONS IN SOUTHERN TURKEY*

Site	Ruscogenin (%) Underground parts	Ruscogenin (%) Aerial parts
Horzum-Kozan/Adana	0.11	0.03
Düziçi/Osmaniye	0.05	0.03
Çamliyayla/Mersin	0.11	0.05
İncebel/Osmaniye	0.12	0.04
Bahçe/Adana	0.02	0.03
Zorkun/Osmaniye	0.07	0.05
Average ± Sx	0.08 ± 0.042	0.038 ± 0.103

***Ruscogenin contents were calculated as ruscogenin + neoruscogenin

RESULTS AND DISCUSSION

Ruscogenin was the main constituent in the extract of *Ruscus aculeatus*. The contents of ruscogenin in the underground and the aerial parts of *R. aculeatus* varied between 0.02-0.12 and 0.03-0.05 %, respectively. In general, ruscogenin contents of underground parts is higher than in the aerial parts except for Bahçe/Adana (Table-2). The present findings showed similar results as reported by Nikolov *et al.*[8] who stated that the content of ruscogenin in the underground and the aerial parts of *R. hypoglossum* were 0.14 and 0.10 %, respectively and for *Ruscus aculeatus* the respective values were 0.12 and 0.08 %. The content of ruscogenin in the aerial parts of samples in the present study are generally lower compare to others in the literature, while the values for underground parts are similar to those cited in the literature. Intra specific variations were found on the comparison of the rate of ruscogenin among the samples of *R. aculeatus*, collected from six different locations. The highest ruscogenin content (0.12 %) was determined in underground parts of *Ruscus aculeatus* collected from Incebel/ Osmaniye (Fig. 2). Akyol[17] used a TLC method to report the ruscogenin content in the underground parts of *R. aculeatus* collected from Bursa-Inegöl in the northeast Anatolian to be around 0.5-0.6 %. Vegetative spread and reproduction by rhizomes and stolons shows a wide spectrum of developmental plasticity. At later stages of growth, involving shifts in rhizome and stolon responses to environmental cues, with concomitant changes in their geotropism and morphology, the developmental plan for rhizome production and shoot growth is predetermined and independent of the environment (autonomous development) as in the case of *Ruscus hypophyllum*[18]. Chemical variation among populations can be attributed to genetic and/or environmental factors[19]. The observed annual differences in plant chemistry ostensibly derive from annual differences in resource

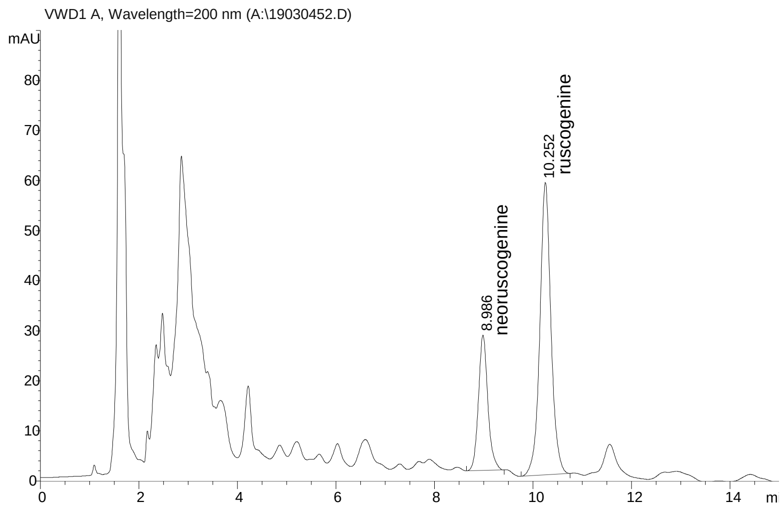

Fig. 2. Chromatogram of ruscogenins in the extract of underground part of *R. aculeatus* collected from Incebel, Osmaniye

availability and environmental conditions, which result in disparate demands on primary and secondary metabolic systems[20]. Chemical phenotypic plasticity is characteristic of plant populations in changing environments[21] and results in the pronounced chemical variation observed in heterogeneous regions[22,23]. Numerous studies have documented plant chemical variation and phenotypic plasticity in plant chemistry in response to environmental change[23-26]. Plants can respond to a changing environment through altered production of myriad primary and secondary metabolites[27]. Additionally, roots are influenced directly by changes in the soil environment[28]. Chemical changes observed in root extracts of individuals of *Caltha leptosepala* and *Trollius laxus* transplanted between environmentally distinct sites revealed that the observed chemical variation was due in part to phenotypic plasticity in response to environmental demands and that response was habitat specific. Root extracts of both species exhibited temporal and spatial variation in chemical composition and antifungal activity. These results underline the importance of habitat characteristics in the expression of plant chemical characters. Additional studies toward understanding chemical variation in plants are important for present understanding of the interface between evolution and ecology[29]. Some morphological and chemical changes in *Ruscus* species can be occurred related to the growing habitats[30]. The fact that the highest ruscogenin rates in the underground parts are obtained at plants collected from the different elevations (500, 650, 1430 m) can be highly attributed to shading ratios and soil humidity in changing environments rather than various elevations (Table-1).

The differences among Ruscogenin rates in the *Ruscus aculeatus* plant collected from Zorkun/Osmaniye, Bahçe/Adana, Incebel/Osmaniye, Düziçi /Osmaniye, Horzum-Kozan/Adana, ÇamLiyayla/Mersin in Turkey can be caused by source of ecological variation at the environmental factors. Plants can respond to changing ecology by varying the production of chemical metabolites. As a result of present studies the ruscogenin contents changed between the limits given in the literature.

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