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Comparative study of three rosemary (*Rosmarinus officinalis* L.) clones during the growing season

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Summary: The growth rate of three rosemary clones, 'Harmat', 'Salem' and 'Horvát', their essential oil content and its components as well as the total phenol content and total antioxidant capacity in their aqueous and ethanolic extracts were compaired. Total phenol content (determined by Folin-Ciocalteau reagent) and antioxidant power (FRAP-value) of aqueous and ethanolic rosemary extracts were measured by spectrophotometric method. The essential oil content was determined from drug by water-steam distillation. 'Harmat' showed the most intensive growth through the vegetation period. The total antioxidant power of the ethanolic extracts from all the three clones was similar: a decrease was observed at the end of the season. In most cases more antioxidant compounds were dissolved in the aqueous extracts. The total quantity of phenolic compounds shows a good correlation with the potential extent of stress effects. The difference among the phenol contents of the various clones can be derived from the difference in their tolerance, because phenols have antioxidant effects contributing to protection against harmful impacts. Difference between the two extraction methods (aqueous and ethanolic) is due to the distinct solubility of compounds. Some other components, like essential oil compounds also possess antioxidant effect and in this way they may influence the antioxidant power of extracts.

Key words: antioxidant capacity, essential oils, phenol, rosemary, *Rosmarinus officinalis*

Introduction

Rosemary (*Rosmarinus officinalis* L.) has been known and used as an aromatic and medicinal plant since the ancient times. This evergreen shrub belongs to the *Labiatae* (*Lamiaceae*) family and some of its medical and physiological effects are partly attributed to its compounds with antioxidant activity (*Edwin* et al., 1996; *Huang* et al., 1996; *Hidalgo* et al., 1998; *Zheng & Wang*, 2001; *Baño* et al., 2003). Among these compounds, the polyphenols form one of the most characteristic groups. A large number of polyphenolic compounds with antioxidant effect have been identified in rosemary. Several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, 7-methylepirosmanol, methyl carnosate have been reported. Besides diterpenes several flavonoids (genkwanin, apigenin, cirsimaritin etc.) and some phenolic acids such as rosmarinic and caffeic acids are also shown to be present among rosemary's active agents (*Shu-Wen Huang* et al., 1996; *Hidalgo* et al., 1998; *Baño* et al., 2003). Nevertheless, antioxidant property of rosemary is not only due to its phenol content. Several essential oil components such as λ-terpinene, estragol, β-pinene etc. and α-tocopherol also possess antioxidant power (*Saricoban & Ozcan*, 2004; *Ruberto & Baratta*, 2000; *Chevolleau*, 1993).

These components are present in most parts of rosemary, but their amounts during the whole developmental process of the plant depend on several parameters such as the stage of the development, weather conditions or any types of unfavourable impacts on the physiological state of plants (*Baño* et al., 2003). Out of the various environmental stress effects, freezing temperature may have a major harmful impact on plants originating from warmer ecosystems (i.a. from the Mediterranean region). Our cultivated plants must endure unfavourable conditions, an ability, which was profoundly examined in frost sensitive crops, like peach and nectarin (*Szabó* et al., 1997), apricot (*Pedryc* et al., 1997) or other stone fruit species (*Szalay* et al., 2004), as well as herbs like rosemary (*Tulok*, 2000). From these species frost tolerant genotypes (like 'Harmat' in the case of rosemary) may have an adventage over those more susceptible to the same effects.

Antioxidant capacity of rosemary extracts may also depend on the extraction method because these components are characterized by different solubility. Some of them are water-soluble such as rosmarinic acid, but some compounds are less hydrophilous such as carnosic acid (*Maike & Monique*, 2003; *Dorman* et al., 2003; *Wada* et al., 2004).

The aims of this study were to reveal developmental differences among three rosemary genotypes and follow the changes in the total antioxidant power and total phenol content in their aqueous and ethanolic extracts during the

whole growing season. Essential oil content and composition of drugs were also determined since it is a very important property from the medical point of view.

Material and method

Plant material

Ten-centimetre-long shoots were harvested at five periods in the growing season of 2003 from three different rosemary (*Rosmarinus officinalis* L.) clones ('Harmat', 'Salem' and 'Horvát'). The origins of these clones are different. 'Harmat' is a Hungarian frost-tolerant cultivar approved in 1999. 'Salem' is a Moroccan and 'Horvát' is a Croatian clone. Half-woody cuttings of the clones were planted at the Experimental Farm of the Department of Medicinal and Aromatic Plants (Faculty of Horticulture, Corvinus University of Budapest, Hungary).

Growth parameters

Three parameters were recorded to characterize the growth behaviour of the clones: height, diameter of rosemary shrubs as well as the length of young shoots were measured six times during the vegetation period.

Extraction methods

The collected plant materials were dried at room temperature and grounded to prepare the adequate extractions. Aqueous (1 g drug /100 ml hot distilled water) and ethanolic (1 g drug/ 100 ml 20% ethanolic) extractions were obtained. The aqueous extracts were filtered after 24 hours of incubation while the ethanolic extracts were filtered after 72 hours.

Determination of total antioxidant capacity and total phenol content

Total antioxidant capacity and total phenol content was measured from the aqueous and ethanolic extracts. The total antioxidant power was assessed by the FRAP method (Ferric Reducing Ability of Plasma) and measured by spectrophotometric way at $\lambda = 593$ nm (*Benzie & Strain,* 1996). The total phenol content was determined by using Folin-Ciocalteau reagent and measured by spectrophotometer at $\lambda = 760$ nm (*Singleton & Rossi,* 1965). Calibration curves obtained by ascorbic acid (in the case of FRAP) and gallic acid (phenol content) were used for the quantitative analyses. The investigations were performed at the Department of Applied Chemistry (Faculty of Food Science, Corvinus University of Budapest, Hungary).

Essential oil content and its components

The plant material for the examination was collected on the 8th May. The essential oil content of the drugs was determined by water-steam distillation of drugs in a Clevenger–apparatus followed the prescriptions of the Ph. Hg. VII. Quantities of the components were determined by gas chromatography. These measurements were carried out at the Department of Medicinal and Aromatic Plants (Faculty of Horticultural Science, Corvinus University of Budapest, Hungary).

Results

Growth rate

The height of 'Harmat' and 'Salem' changed almost in parallel during the whole vegetation period *(Figure 1)*. Their rate of growth was quite steady. A considerable difference in the growth patterns was obvious toward the end of the growing period. At the end of the summer 'Harmat' showed an intensive growth. Its height (49.8 cm) measured at the end of the growing season was significantly higher than that of 'Salem'. The growth intensity of 'Horvát' was always under the values measured for the other two clones, its height reached 25.5 cm at the last measuring time.

The results concerning the diameter of the shrub were similar in the case of 'Salem' and 'Horvát' *(Figure 2)*. Significant difference between them could only be observed in the middle of August, when the shrub diameter of 'Horvát' (28.9 cm) was exceeded by that of 'Salem'

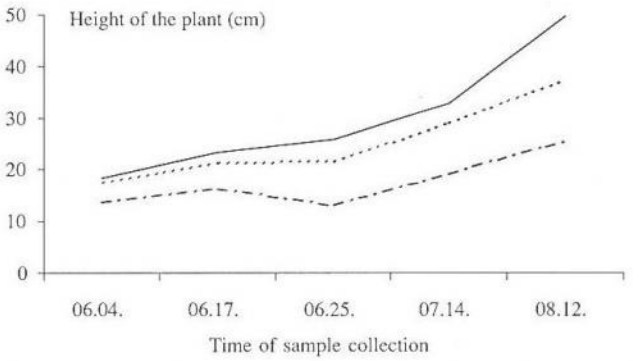

Figure 1 Height of the rosemary clones at different times in the growing season (Soroksár, 2003). — 'Harmat', ····· 'Salem', – · – · 'Horvát'

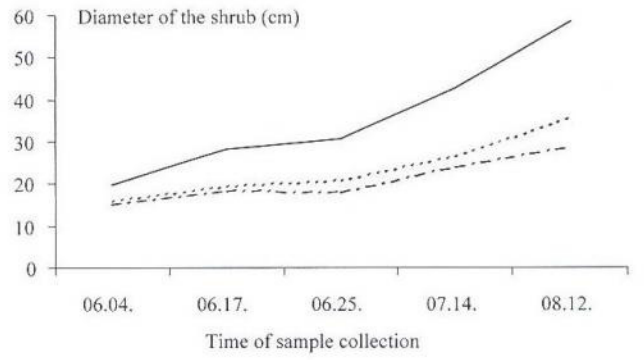

Figure 2 Diameter of shrubs of the rosemary clones at different times in the growing season (Soroksár, 2003). — 'Harmat', ····· 'Salem', – · – · 'Horvát'

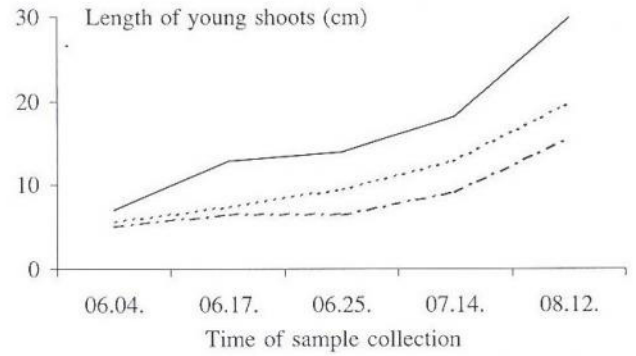

Figure 3 Length of the young shoots of rosemary clones at different times in the growing season (Soroksár, 2003). — 'Harmat', ····· 'Salem', – · – · 'Horvát'

(35.6 cm). The shrub diameter of 'Harmat' was constantly greater than that of the two other genotypes. This difference was the most considerable at the end of the season.

Length of the young shoots of each clone reflected different growing intensities. The biggest difference was measured at the end of the growing season *(Figure 3)*. 'Harmat' showed the most intensive growth, while 'Horvát' could be characterized as the less intensively growing genotype. According to our results, the highest growth rate was measured in the case of 'Harmat', which was followed by the selections 'Salem' and 'Horvát'.

Antioxidant properties of aqueous and ethanolic extracts

Total phenol content measured from the aqueous and ethanolic extracts of 'Harmat' was relatively invariable during the whole season *(Figure 4A)*. The measured values fell within the range between 0.205 mg/ml and 0.295 mg/ml. Total antioxidant power of the aqueous extracts obtained at different times during the examined season was also quite similar at 0.50-0.77 mg AA/ml *(Figure 4B)*. Nevertheless, the antioxidant capacity of the ethanolic extract was considerably reduced toward the middle of July (0.138 or 0.065 mg AA/ml).

In the total phenol content of aqueous extracts from 'Salem', an inconsiderable fluctuation was observed: a slight increase was followed by a minor decrease *(Figure 5A)*. The highest total phenol content was measured at the end of June (0.558 mg/ml), while in the case of the alcoholic extracts, total phenol content reflected a slight, but steady increase. The highest value (0.682 mg/ml) was registered in the middle of August. The total antioxidant capacity was nearly constant in both extracts, although a small fluctuation could be observed *(Figure 5B)*. Aqueous extracts showed a maximum value (0.939 mg AA/ml) at the middle of July, while in ethanolic extracts a slight decrease was observed (0.466 mg AA/ml) at the middle of August.

The changes in the total phenol content of both extracts made from 'Horvát' can be described by a tendency similar to that obtained for 'Salem' *(Figure 6A)*. Phenol content of the aqueous extract increased toward the end of June, and

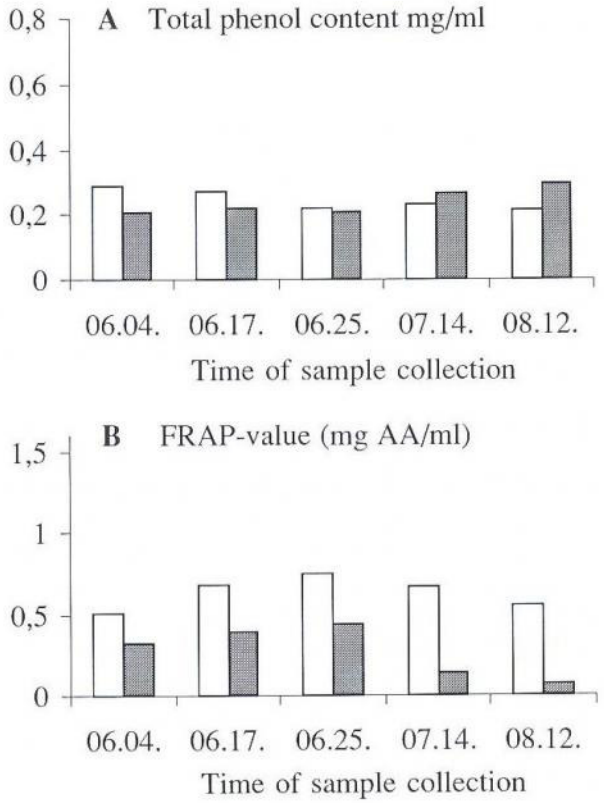

Figure 4 Total phenol content (A) and total antioxidant capacity (B) of the water and ethanol extracts from Rosmarinus officinalis 'Harmat' during the growing season (Soroksár, 2003). □ Water extract, ■ Ethanol extracts

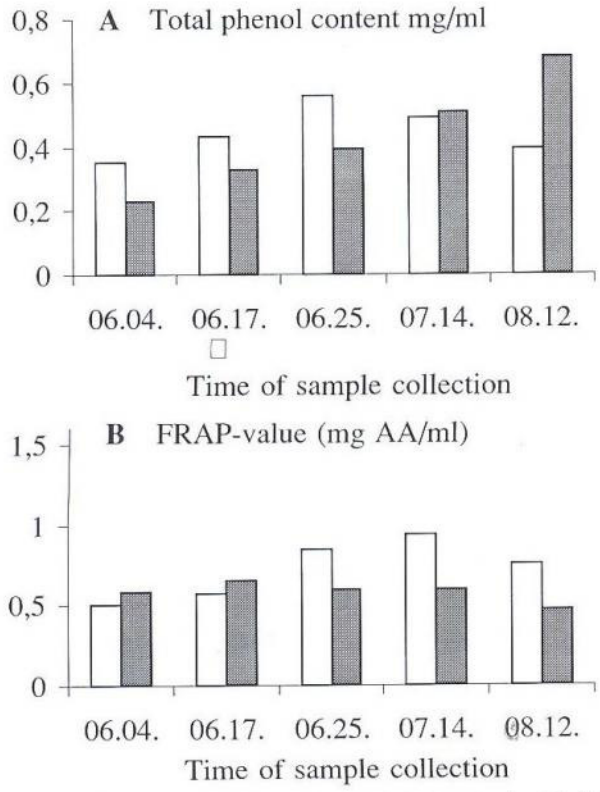

Figure 5 Total phenol content (A) and total antioxidant capacity (B) of the water and ethanolic extracts from Rosmarinus officinalis 'Salem' during the growing season (Soroksár, 2003). □ Water extract, ■ Ethanol extracts

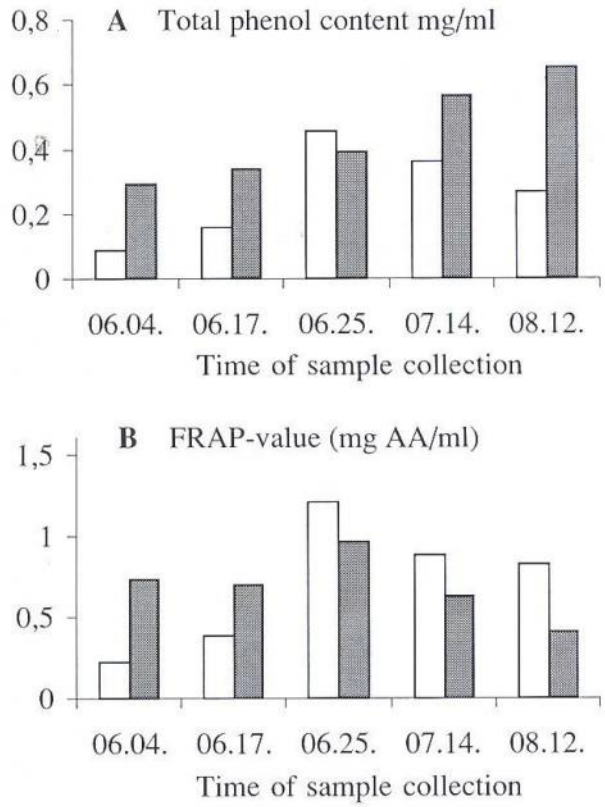

Figure 6 Total phenol content (A) the total antioxidant capacity (B) of the water and ethanolic extracts from Rosmarinus officinalis 'Horvát' during the growing season (Soroksár, 2003). □ Water extract, ■ Ethanol extracts

then it began to decrease. But the degree of this change was greater than that measured from extracts of 'Salem'. The ethanolic extracts reflected a substantial increase as in the case of 'Salem'. The total antioxidant power fluctuated in a similar way in both extracts *(Figure 6B)*. The FRAP-values increased toward the end of June, and then they started to decrease. The lowest antioxidant capacity was shown at the middle of August. These changes were more definite in the aqueous extract.

'Salem' and 'Horvát' had significantly higher antioxidant power and total phenol content than 'Harmat'. In aqueous extracts, the total phenol content of 'Harmat' was constant during the whole season while in the case of 'Salem' and 'Horvát' a similar tendency of fluctuation was observed with a maximum value at the end of June. The total phenol content measured from the ethanolic extract of 'Harmat' was also constant, while the values for the other two clones changed in a similar way: after a continuous increase reached their maximum at the middle of August.

The total antioxidant capacity of the aqueous extracts from 'Hamat' and 'Salem' was very similar. 'Horvát' showed a considerable maximum value at the end of June. Total antioxidant power of the ethanolic extracts from all the three clones was somewhat similar: a decrease was observed at the end of the season.

Differences among the measured values were not only evaluated in function of the various rosemary genotypes but also according to the different extraction methods. Taking into consideration that aqueous and ethanolic extractions were carried out from three genotypes at five different periods, phenol content of aqueous extractions was higher in 7 cases out of the total 15 cases. Total antioxidant capacity was higher in the aqueous extracts in 11 samples, especially 'Harmat'.

Amount and components of essential oil

The antioxidant activity can also be influenced by some of the secondary metabolic products. Among these products, several essential oil compounds have outstanding importance. The essential oil content of the clones was determined at the beginning of May *(Figure 7)*. At that time, the essential oil contents of 'Harmat' (1.40 ml/100g) and 'Horvát' (1.09 ml/100g) were nearly identical. They reached the value prescribed by the Hungarian Standard. 'Salem' was very poor in this respect (0.21 ml/100g).

To gain further insight for the comparison of the three tested clones, the essential oil composition was also determined *(Table 1)*. Camphor (18.3%) and 1,8-cineol (16.9%) were the main compounds of 'Harmat'. Besides them, α-pinene (9.7%), β-pinene (9.7%) and bornylacetate (8.7%) were also present in a considerable amount.

'Salem' had three characteristic components: bornylacetate (25.4%), borneol (17.4%) and camphor (15.6%). Besides them, 1,8-cineol (9.6%) and β-caryophyllene (7.1%) were contained in greater amounts. Other components were found just in traces and α-pinene was completely missing from its essential oil content.

Camphor was present in an outstanding amount (48.6%) constituting nearly the half of the total essential oil content of 'Horvát'. The quantity of 1,8-cineol is also worth mentioning, which was found in the same amount as in 'Salem'. Three components, including β-myrcen, octen-3-on and β-caryophyllene were absent from the volatile oil of 'Horvát'.

Briefly, 1,8-cineol and camphor were present in a considerable (but not the same) amount in the essential oil of the three clones. Considering the other components, the tested clones showed different tendencies

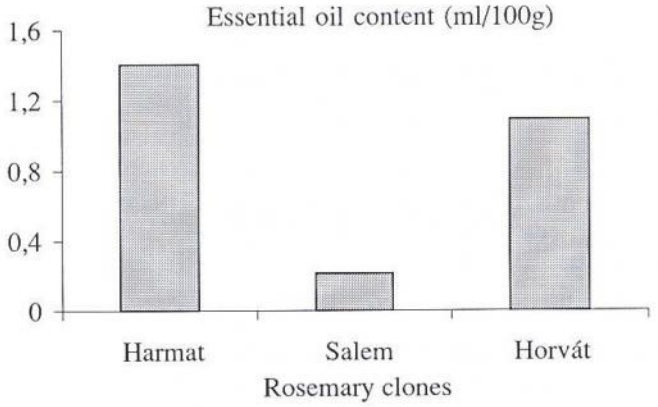

Figure 7 Amount of essential oil in the drugs of 'Harmat', 'Salem' and 'Horvát' rosemary clones (Soroksár, 2003)

Table 1 Essential oil composition (%) of 'Harmat', 'Salem' and 'Horvát' rosemary clones (Soroksár, 2003)

Essential oil components of rosemary clones (%)			
Components	'Harmat'	'Salem'	'Horvát'
borneol	6.4	17.4	2.4
bornylacetate	8.7	25.4	2
camphen	4.9	0.3	2.7
camphor	18.3	15.6	48.6
β-caryophyllene	3.7	7.1	–
1,8-cineol	16.9	9.6	9.6
estragole	1.9	2.2	1.9
β-myrcen	1.5	0.4	–
octen-3-on	1.8	0.4	–
α-pinene	9.7	–	4.3
β-pinene	7.7	0.8	2.6
λ-terpinen	2	1	1.7
verbenon	3.7	1	5
others	12.8	18.8	19.2

Discussion

The difference among the tested clones considering their growth rate or phenol content and antioxidant capacity of their extracts, as well as essential oil content and composition can be explained by several reasons. All these parameters are presumably genetically encoded properties, but they can be modified by various external or internal factors.

Phenolic compounds are one of the main antioxidant components of rosemary. Fluctuations in their amount can be caused by environmental factors like heat stress or solar radiation (*Baño* et al., 2003). In the case of 'Salem' and 'Horvát', phenol content increased during the summer and it reached a maximum level at the middle of August, when heat and solar radiation was the most expressed. Differences in the phenol content of ethanolic and aqueous extracts can be explained by the different phenol types being present in rosemary (*Edwin* et al., 1996; *Huang* et al., 1996; *Hidalgo* et al., 1998; *Zheng & Wang,* 2001; *Baño* et al., 2003). The majority of the phenols are non-polar compounds (carnosic acid, carnosol etc.), which explains why their quantity was higher in most of the ethanolic extracts. Total quantity of phenolic compounds shows a good correlation with the potential extent of stress effects. The difference among the phenol contents of the various clones can be derived from the difference in their tolerance, because phenols have antioxidant effects contributing to the protection against harmful impacts. As a consequence of several stress effects (low temperature, extreme solar radiation, heavy metal pollution etc.), phenol content increases in plant tissues (*Stefanovits-Bányai* et al., 2002). The more sensitive plants need higher phenol content. 'Harmat' is a frost-tolerant species, which showed lower phenol content compared to those of the two other clones. Various phenol contents of the different extracts may partly be attributed to dissimilar secondary metabolic processes in the tested plants. At the time of sample collection, the rate of secondary metabolic processes like biosynthesis of phenols may differ in tissues of the investigated rosemary clones. Varied levels of total phenol content may reflect these biochemical processes.

Although phenolic compounds have profound antioxidant effects (*Hidalgo,* 1998; *Bano,* 2003; *Dorman* et al., 2003), in some cases total antioxidant power and total phenol content of extracts showed diverse tendencies. The reason for this is that besides phenols rosemary contains several components, which could also be dissolved in the extracts. Some of these constituents have antioxidant capacity (vitamins, essential oil compounds etc.) and in this way they can increase the antioxidant power of extracts (*Saricoban & Ozcan,* 2004; *Ruberto & Baratta,* 2000; *Chevolleau,* 1993). On the other hand, there may be also other type of components, which can decrease the antioxidant effect.

Difference between the two extraction methods (aqueous and ethanolic) is due to the different solubility of compounds. In most cases, more antioxidant compounds were dissolved in the aqueous extract (*Maike & Monique,* 2003; *Dorman* et al., 2003; *Wada* et al., 2004).

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