RESEARCH ON THE FLAVONES CONTENT IN FOUR SPECIES OF MEDICINAL PLANTS GROWN IN THE WESTERN TRANSYLVANIAN PLAIN

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Abstract. *Extracts of four species of Lamiaceae family medicinal plants (Agastache Foeniculum, Nepeta cataria, Melissa officinalis and Lavandula angustifolia) used in phytotherapy were screened for flavone content in two different harvest periods and two different hours of the day. Through this research we want to emphasize the optimal harvest time of vegetal raw material, when the studied plants contain the maximum active principles. The content of flavone/flavonols, main phenolics contained by these plants, was determined spectrophotometrically, according to Dowd method in APHIS Laboratory from USAMV Cluj-Napoca, with a Synergy 2 spectrophotometer, microplate reader. Duplicate samples of each plant were harvested for determinations from raw plant (fresh) and dry material. Following the data from Agastache Foeniculum and Nepeta cataria, the highest flavone content was obtained from the raw material harvested on 09/06/2014, at 16 hour, in both fresh and dried raw material. In the Melissa officinalis species, the highest flavone content was found in raw material (fresh and dry) which was harvested on 09/06/14, 11 hour. The highest flavone content in Lavandula angustifolia was found on 19/06/2014, 11 hour, in the fresh material and at 16 hour in the dry one. For the four studied species, the obtained results regarding the flavone content show that the optimal time of harvest was 09/06/2014, ie at the beginning of flowering, except for the species Melissa officinalis for which the flowering occured after 20/06/2014.*

Keywords: *Agastache foeniculum, Nepeta cataria, Melissa officinalis, Lavandula angustifolia, polyphenols.*

INTRODUCTION

Dietary antioxidants have a great importance for the prevention of different diseases or health improvements and herbal preparations are a rich source of these compounds (Blasa et al., 2010; Bobiş et al., 2014; Turkmen et al., 2005).

Plant extracts obtained with different techniques and solvents are mixtures of several classes of compounds, rich in biologically active principles, represented by secondary metabolites synthesized in their growing process. The main bioactive compounds in plants are phenolic acids, free and glycosilated flavonoids (flavones, flavonols, dihydroflavones, flavan-3-ols etc.).

Lamiaceae family provide more than 200 plants genera. Special interest have *Lavandula angustifolia*, *Melissa officinalis*, *Nepeta cataria* and *Agastache foeniculum* for their high content of bioactive compounds.

Lavandula angustifolia is the only member of *Lavandula* genus which is recognized officially as a medicinal plant and used in phytotherapy (BLAŽEKOVIĆ et al., 2010). The chemical composition of this plant have been subjected to several studies over the past years, studies focused on the organic extracts, fractions of these extracts or essential oils extracted

from aerial parts or flowers of the plant (BLAŽEKOVIĆ ET AL., 2010; HAJHASHEMI ET AL., 2003; HOHMANN ET AL., 1999; LIS-BALCHIN AND HART, 1999).

Melissa officinalis, popularly known as lemon balm is a medicinal plant having different properties in traditional medicine (treatment of gastrointestinal disturbance, minor sleeplessness, antioxidant and neuroprotective effects)(HANGANU ET AL., 2008; DUDA ET AL., 2007; ONIGA et al., 2010).

Nepeta cataria (catnip) leaves possess pleasant minty and lemony scent and taste and has been used in traditional medicine since long time. Also catnip is used for flavouring sauces, soups and cooked foods. It is used in phytomedicine as antispasmodic, antitussive, sedative, carminative, stomachic and tonic (DUKE, 1987; MIHAYLOVA et al., 2013). *Nepeta cataria* var. *citriodora* is mentioned as a possible adulteration or substitute for lemon balm (Melissa officinalis)(MODNICKI et al., 2007).

Agastache foeniculum is a herbaceous species, perennial, used in ornamental, medicinal and aromatic purposes (DUDA et al., 2013), known under the name "gyant hyssop". Among all medicinal plants, *A.foeniculum* presents an increasing interest in traditional phytotherapeutics, due to the essential oils composition and also non-volatile metabolites (ZIELINSKA AND MATKOWCKI, 2014).

The main objective of the present paper is to present the flavone content in the aerial parts from four species of *Lamiaceae* family (medicinal and aromatic plants) harvested in four different periods grown in the experimental field of UASVM Cluj-Napoca from Jucu.

AIMS

Through this research we wanted to emphasize the optimal harvest time of vegetal raw material, moment in which the maximum level of active principles are produced.

MATERIALS AND METHODS

The flavone/flavonol content of ethanolic extracts from *Agastache foeniculum, Nepeta cataria, Melissa officinalis* and *Lavandula angustifolia* was determined. These species of perennial medicinal plants were cultivated for approx. 7 years in the experimental field from Jucu of our university.

Plant samples were harvested in four steps, during two phenological periods: 9 and 19 of June 2014, in two different times of the day (11.00 and 16.00 hours).

Duplicate samples of each four species were collected - one for determination of active principles from fresh material, and the second sample was dried protected from light, and then analyzed.

Active principles determination from ethanolic extracts (2% in ethanol 70%) was carried out in APHIS Laboratory of USAMV Cluj-Napoca, using a Synergy 2 spectrophotometer, micro plate reader. Flavone/flavonol determination was performed following the Dowd method (DOWD, 1959), with some modifications.

A solution of aluminum chloride (2% in methanol) is used as a specific reagent for flavone/flavonol determination. In the presence of flavonoids the reagent develops a yellow-orange colour, proportionally with the amount of the compounds. The intensity of this color can be determined spectrophotometrically at 415 nm.

Work technique: a dilute ethanolic solution of each sample (100 μL) was mixed with the same amount (100 μL) of aluminum chloride 2% in methanol. Absorbance was recorded after 10 minutes of reaction. For flavone/flavonols quantification, a calibration curve of

different dilutions of quercetin (0.005 – 0.1 mg/mL) was performed (y=7.435x+0.049, r^2=0.997).

Interpretation of the results for active principles (flavones) was performed by analysis of variance, Student's t test and Duncan test and that the two-factor statistical calculation program « ANOVA» of the discipline Experimental Techniques in our university.

RESULTS

Flavones and flavonols are plant derived polyphenolic compounds useful in human diet. They are presented in fruits, vegetables, tea, red wine and medicinal and aromatic herbs. Most flavonoids are known to be chemo-preventive, lowering risks of cancer and other diseases. Medicinal plants from *Lamiaceae* family are known to have high amounts of polyphenols in general and consequently flavonoids also. The main problem for harvesting these benefic plants is finding the optimum moment, when these compounds have the highest accumulation in different parts of the plant. The present study tried to find this optimal moment, by harvesting four species of *Lamiaceae* family in two different phonological stages, and in two different hours of the day. Results show that these four periods show different amounts of flavones, and give the answer regarding the optimal moment of harvest, in order to have the highest amount of active principle.

Tables 1 and 2 present the results of flavone content from *Agastache foeniculum* (extract made from fresh and dry plant).

Table 1

Flavone content of fresh aerial parts from *Agastache foeniculum*

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	9.42	100.0	0.00	Control	A
	16	10.99	116.8	1.58	***	C
19/06/2014	11	10.95	116.3	1.53	***	C
	16	10.22	108.5	0.80	***	B
DL (p 5%) = 0.35; DL (p 1%) = 0.48; DL (p 0.1%) = 0.68						

Table 2

Flavone content of the dried aerial parts from *Agastache foeniculum*

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	16.40	100.0	0.00	Control	B
	16	19.06	116.2	2.66	***	C
19/06/2014	11	15.89	96.9	-0.52	00	A
	16	16.30	99.4	-0.10	-	B
DL (p 5%) = 0.35; DL (p 1%) = 0.48 ; DL (p 0.1%) = 0.68						

Obtained results were in accordance with literature study findings, even if the extraction methods and the way of results expression were sometimes different (DAPKEVICIUS ET AL., 1998; ZIELINSKA AND MATKOWCKI, 2014).

Positive very significant differences regarding the content of flavones from dry material, compared to the control (sample harvested 09/06/2014, 11 hours) was obtained from raw materials harvested on 09/06/2014, 16 hours.

Interpreting the same results also through the multiple comparisons method (Duncan test) it can be observed that the highest content of flavanols is obtained from raw materials harvested in early flowering, at 16 hours.

The content of flavones from *Nepeta cataria* (catnip) is presented in tables 3 and 4.

Through the analysis of variance is established a very significant difference in fresh material and distinct significant in dry material compared to the control (09/06/2014, 16 hours) regarding the content of flavones.

By interpreting the data using the Duncan test we found that the best time to harvest is the beginning of flowering (for the last year, 9th of June), in the afternoon (16 hours), both for fresh and dry material.

Table 3

Flavone content of fresh aerial parts from *Nepeta cataria* (catnip)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	2.64	100.0	0.00	Control	C
	16	2.99	113.2	0.35	***	D
19/06/2014	11	1.80	68.2	-0.84	000	A
	16	1.89	71.3	-0.76	000	B
DL (p 5%) = 0.07; DL (p 1%) = 0.10; DL (p 0.1%) = 0.16						

Table 4

Flavone content of the dried aerial parts from *Nepeta cataria* (catnip)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	8.07	100.0	0.00	Control	B
	16	8.48	105.0	0.40	**	C
19/06/2014	11	5.91	73.2	-2.17	000	A
	16	6.05	74.9	-2.03	000	A
DL (p 5%) = 0.18; DL (p 1%) = 0.27; DL (p 0.1%) = 0.44						

Centralization and interpretation of statistical data on the content of flavones from *Melissa officinalis* (lemon balm) is shown in Tables 5 and 6.

Through the analysis of variance is established a significant very negative difference at all moments of harvest compared to the control (09/06/2014, 11 hours) for fresh raw material. In terms of dry sample material differences are significantly negative for the samples collected on 9 and 19/06/2014, 16 hours, compared to control (09/06/2014, 11 hours).

The highest content of flavones is in the raw material (fresh and dry) harvested at the beginning of flowering (09/06/2014), 11 hours.

Table 5

Flavone content of fresh aerial parts from *Melissa officinalis* (lemon balm)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	2.94	100.0	0.00	Control	D
	16	2.40	81.5	-0.54	000	B
19/06/2014	11	2.56	87.2	-0.38	000	C
	16	2.23	75.9	-0.71	000	A
DL (p 5%) = 0.07; DL (p 1%) = 0.11; DL (p 0.1%) = 0.18						

Table 6

Flavone content of the dried aerial parts from *Melissa officinalis* (lemon balm)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
09/06/2014	11	5.93	100.0	0.00	Control	B
	16	5.49	92.5	-0.44	0	A
19/06/2014	11	5.60	94.5	-0.33	-	AB
	16	5.55	93.6	-0.38	0	A
DL (p 5%) = 0.34; DL (p 1%) = 0.51; DL (p 0.1%) = 0.82						

In Tables 7 and 8 are centralized and interpreted statistical data on the content of flavones from *Lavandula angustifolia*.

It can be observed very low concentrations of flavones, with significant negative differences compared to the control (09/06/2014, 11 hours) to fresh aerial parts. In contrast to fresh feedstock, at the dry matter content it is noticed a very high concentration in flavones on 09/06/2014, 16 hours, with positive significant differences compared to the control.

By statistialy interpreting the data using the Duncan test it appears that the optimal time for harvesting samples is at early flowering (last year was 9th of June), before noon, at 11 hour, in the case of fresh ones and in the afternoon, at 16 hours in the case of raw dried material regarding the content of flavones.

Table 7

Flavone content of fresh aerial parts from *Lavandula angustifolia* (lavender)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
06/09/2014	11	1.75	100.0	0.00	Control	D
	16	1.62	92.6	-0.13	000	B
06/19/2014	11	1.66	94.7	-0.09	000	C
	16	1.39	79.7	-0.35	000	A
DL (p 5%) = 0.03; DL (p 1%) = 0.05; DL (p 0.1%) = 0.08						

Table 8

Flavone content of the dried aerial parts from *Lavandula angustifolia* (lavender)

Date	Time of harvest	Concentration mg/ml	Differences		Significance	Duncan Test
			%	Concentration mg/ml		
06/09/2014	11	3.11	100.0	0.00	Control	B
	16	3.61	116.1	0.50	***	D
06/19/2014	11	3.39	109.2	0.28	**	C
	16	2.90	93.2	-0.21	0	A
DL (p 5%) = 0.16; DL (p 1%) = 0.24; DL (p 0.1%) = 0.39						

The concentrations of flavones for the 4 species in the four moments of sampling set according to the research protocol, such as the production of fresh and dry matter, is shown in Figures 1 and 2.

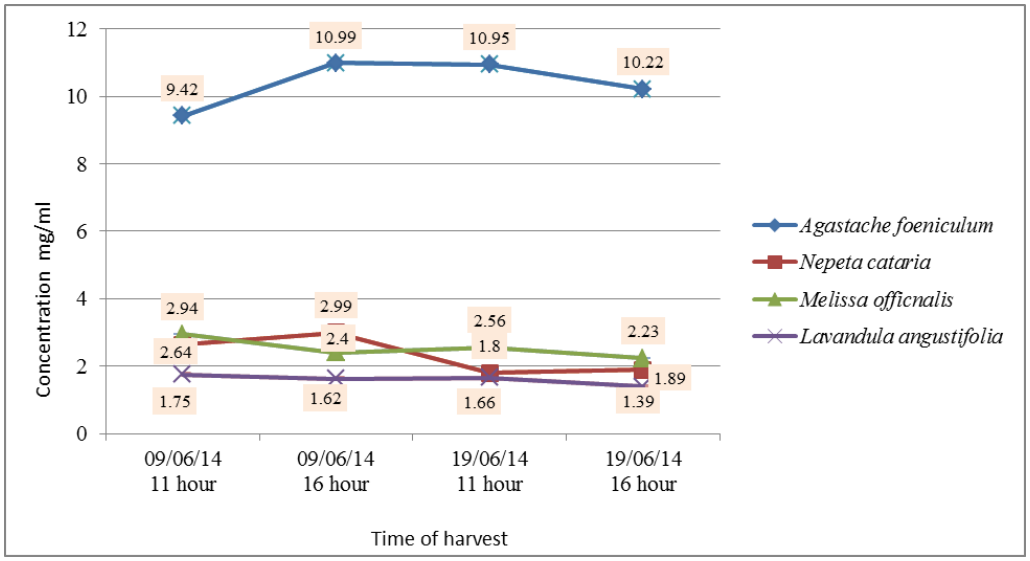

Fig. 1. Flavones content in the fresh raw material of the four studied species

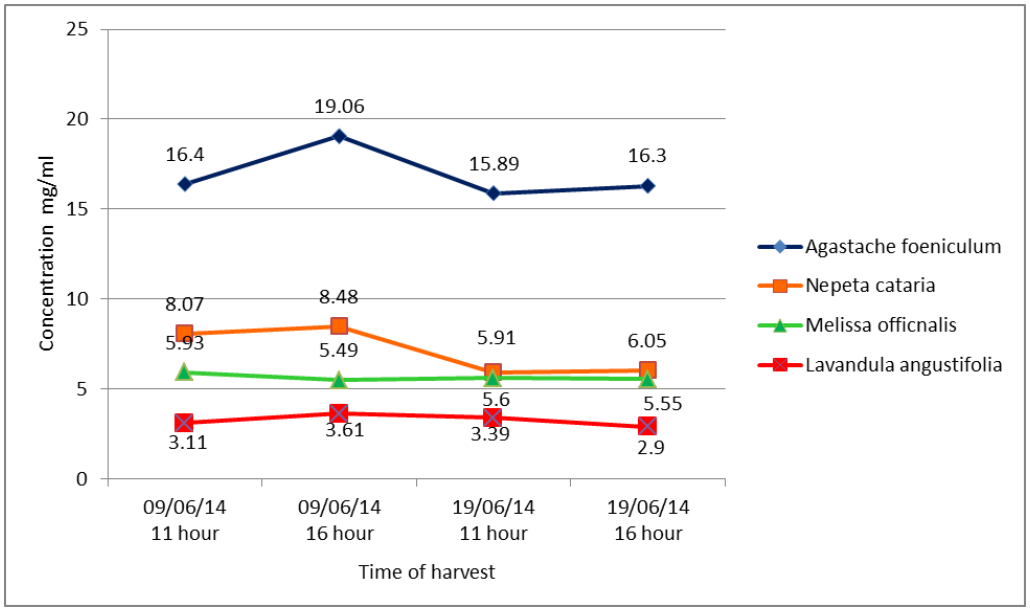

Fig. 2. Flavones content in dry matter raw material of the four studied species

Following the data from *Agastache foeniculum* and *Nepeta cataria* we can conclude that the highest flavone content was obtained from the raw material harvested on 09/06/2014, at 16 hours, in both fresh and dried raw material. In the *Melissa officinalis* species, the highest flavone content was found in the raw material (fresh and dry) which was harvested about 10 days before blooming on 09/06/14, 11 hour. The highest flavone content in *Lavandula angustifolia* was found at the starting period of blooming, in the case of 2014 on the 9th of June, 11 hour, for the fresh material and at 16 hour for the dry one.

CONCLUSION

The optimal harvest time, when the flavones concentration was highest, was found to be the beginning of flowering (09/06/2014), at 16 hour in the afternoon, in three from the four species studied, ie *Agastache foeniculum*, *Nepeta cataria* and *Lavandula angustifolia*, except *Melissa officinalis* species, for which the maximum concentration in flavones was the same date (09/06/2014), earlier in the morning, at 11 hour. On this date lemon balm was approx. 10 days before the starting of flowering.

Of the four species analyzed, the highest concentrations in flavones were recorded at *Agastache Foeniculum*, of 3-5 times higher than in other species, both at fresh and dry material.

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