

POLYPHENOLS ANALYSES FROM *THYMUS* SPECIES

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In this paper we proposed a comparative analyses of polyphenolic compounds from the medicinal products obtained from two species of *Thymus* - *T. vulgaris* L. and *T. comosus* Heuff. From *Thymus* genus, *Thymus vulgaris* L. is onlyest species cultivated as aromatic plant and *Thymus comosus* Heuff. is one of the most important spontaneous and endemic species from Carpat. For these vegetal products, the content of phenyl-propane derivatives and flavonoids was established by using spectrophotometric methods. There were analyzed qualitatively and quantitatively flavonoids and phenyl-propane derivatives, by using HPLC methods, before and after the hydrolysis of extracted compounds.

Key words: *Thymus vulgaris* L.; *Thymus comosus* Heuffe.; HPLC; Polyphenols.

INTRODUCTION

T. vulgaris L. (garden thyme). and *T. comosus* Heuff. belong to *Lamiaceae* family. In Romania, *Thymus* genus contains one cultivated species and 18 wild species. *Thymus vulgaris* L. is only species cultivated as aromatic plant and *Thymus comosus* Heuff. is one of the most important spontaneous species.

The constituents of these species are: volatile oil with a variable content (thymol, methylchavicol, cineol, borneol), flavonoids, phenyl-propane derivatives, tannins.

The thyme volatile oil is strongly antiseptic, the main constituent – the thymol, in particular, is a most effective antifungal. The oil is also an expectorant, it expels worms and have tonic effect, supporting the body's normal function and countering the effects of aging. Thymol, methylchavicol and flavonoids relieve muscle spasms.¹⁻⁶

MATERIALS AND METHODS

There were analyzed the vegetal products represented by the aerial parts (*herba*) obtained from two species of *Thymus* (*Lamiaceae*): *Thymus vulgaris* L., *Thymus comosus* Heuff.

Spectrophotometric determinations were made using a UV-VIS JASCO V-530 spectrophotometer.

The quantitative analysis of flavonoids was made using a method described in the Romanian Pharmacopoeia Xth Edition for the drug *Cynarae folium*.⁸

The quantitative analysis of phenyl-propane derivatives (caffeic acid derivates) was made using the method described in Romanian Pharmacopoeia IXth Edition for the drug *Cynarae folium*.⁷

HPLC determinations⁹

Aparatus and chromatographic conditions: There were used an Agilent 1100 HPLC Series (Agilent, USA) equipped with a degasser G1322A, a quaternary gradient pump G1311A, an autosampler G1311A, a column oven G1316 A, a Zorbax SB-C18 reversed-phase analytical column 100 mm × 3,0 mm *i.d.*, 3,5 μm particle (Agilent, USA) operated at 48°C. The mobile phase was a binary gradient: methanol and

buffer solution. The buffer solution was prepared from KH_2PO_4 40 mM solution in water and the pH was adjusted to 2,3 with 85% ortho-phosphoric acid. The gradient begun with linear gradient from 5% methanol to 42% methanol over the first 35 minutes, followed by isocratic elution with 42% methanol over the next 3 minutes. The flow rate was 1ml/min and data were collected at 330 nm. The injection volume was 5 μ l.

Detection: UV detector at 330 nm. All compounds were identified by comparison of their retention times with those of the standards.

Samples preparation: 30.00 g dried vegetal product were degreased with dichloromethan in the Soxhlet apparatus until the extractive solution became colorless. Then, the vegetal product was extracted with methanol in the Soxhlet apparatus for 4 hours. The methanolic solution was concentrated under reduced pressure at 35°C and the remaining residue was dissolved in 100 ml hot water. After cooling, three extractions were performed in the separating funnel, with ethyl ether, ethyl acetate and 1-butanol. The solvents were removed under reduced pressure and the residues were diluted in methanol. These methanolic solutions of the reuniting residue were analyzed by HPLC.

In order to study the flavonoid aglycons a hydrolysis with HCl 2 N, at boiling for 40 minutes, was performed on each extract.

Standards: caftaric acid, gentisic acid, caffeic acid, chlorogenic acid, p- coumaric acid, ferulic acid, sinapic acid, cichoric acid, hiperozid, isoquercitrin, rutozid, quercitrin, quercetol, patuletin, luteolin, kaempferol and apigenin.

RESULTS AND DISCUSSIONS

For *Thymus vulgaris* L., the content in flavonoids and phenyl-propane derivatives (Table 1) were comparative with those from literature and these

show a good quality of the studied vegetal material. For *Thymus comosus* Heuff., the content of flavonoids is higher than those in *Thymus vulgaris* L. and the content of phenyl-propane derivatives is very closed.

Table 1

Results of spectrophotometrical determinations

Species	Flavonoids (% rutosid)	Phenyl-propane derivatives (% caffeic acid)
<i>Thymus vulgaris</i> L.	0,485	3,57
<i>Thymus comosus</i> Heuff.	0,448	6,70

In Figures 1–4 are presented the HPLC chromatograms of external standards, *Thymus vulgaris* L. extract before the hydrolysis, *T. vulgaris* L. extract after hydrolysis, *T. comosus* Heuff. extract after hydrolysis.

There were used follow standards: 1 – caftaric acid (tR3,27), 2 – gentisic acid (tR 3,76), 3 – caffeic acid (tR 6,10), 4 – chlorogenic acid(tR 6,80), 5 – p-coumaric acid (tR 9,49), 6 – ferulic acid (tR12,80), 7 – sinapic acid(tR15,01), 8 – cichoric acid (tR 15,83), 9 – Hiperozid(tR19,32), 10 – Isoquercitrin (tR 20,27), 11 – Rutozid (tR 20,78), 12 – Miricetol(tR 21,2), 13 – Fisetin (tR 23,1), 14 – Quercitrin (tR 23,64), 15 – Quercetol (tR 27,57), 16 – Patuletin (tR 29,39), 17 – Luteolin (tR 29,93), 18 – Kaempferol(tR 32,50), 19 – Apigenin (tR 33,95).

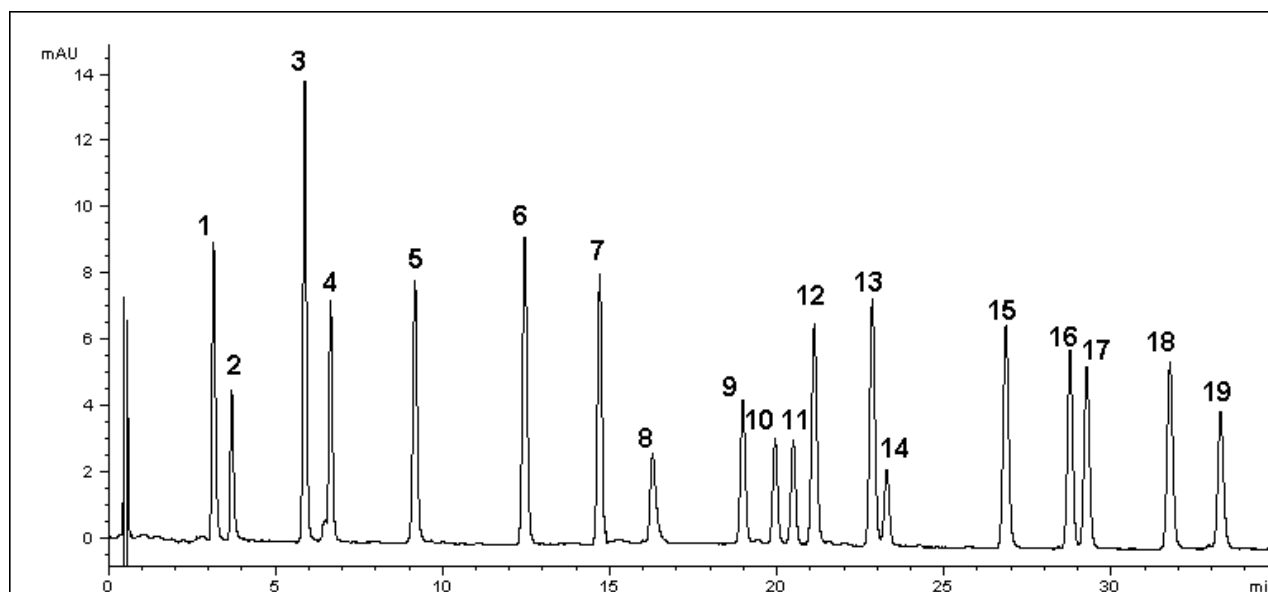


Fig. 1. Chromatogram HPLC of external standards (UV 330 nm).

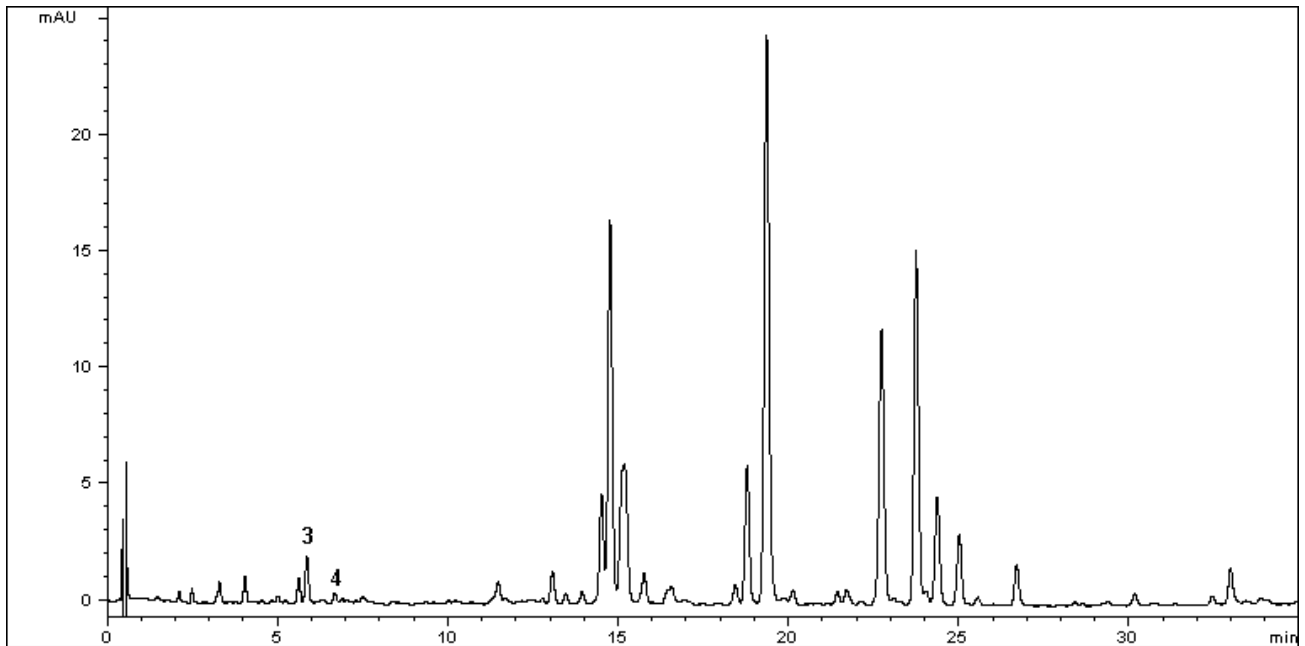


Fig. 2. Chromatogram HPLC of *T. vulgaris* L., before hydrolysis.

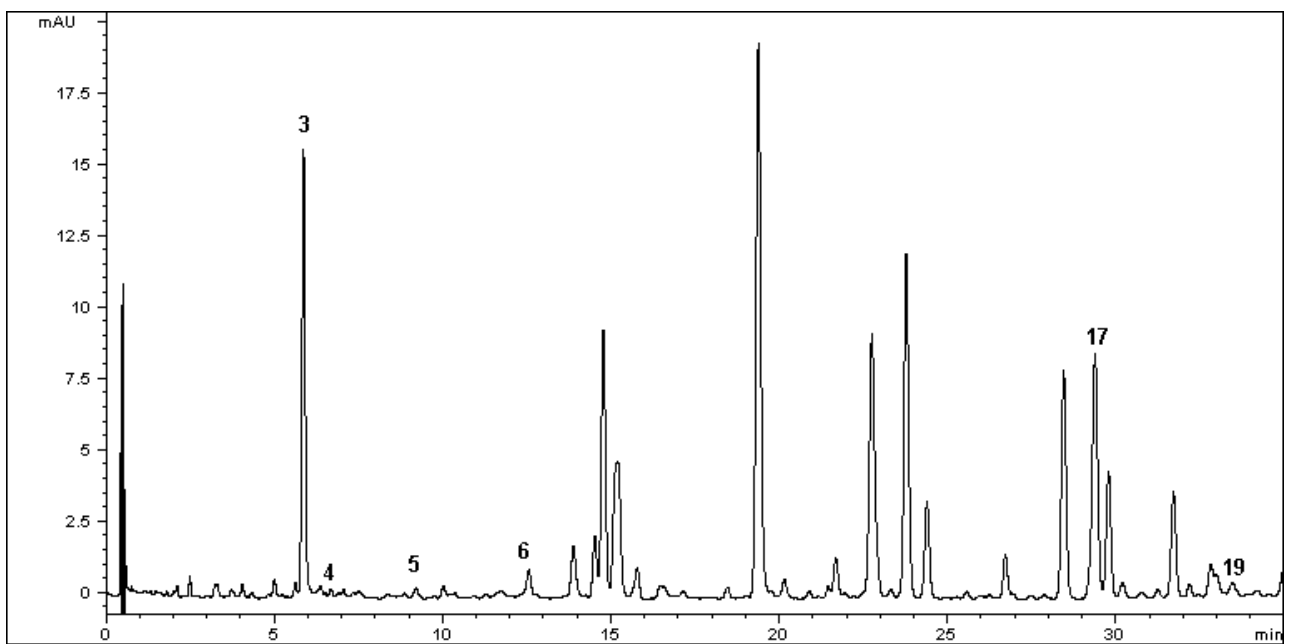


Fig. 3. Chromatogram HPLC of *T. vulgaris* L. after hydrolysis.

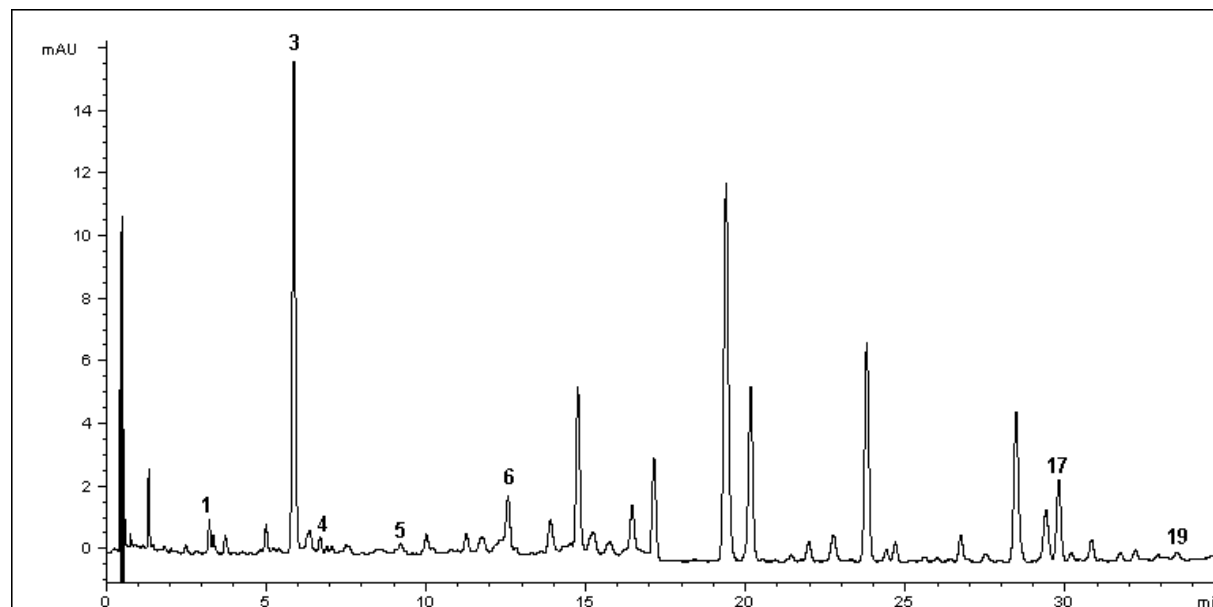


Fig. 4. Chromatogram HPLC of *T. comosus* Heuff. after hydrolysis.

There were identified 7 polyphenolic compounds: caftaric acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin. The quantification of the identified polyphenols was made based on calibration curves. For this compounds the parameters of calibration curves are presented in Table 2.

The concentration of each identified compound from the herba of *T. vulgaris* L. and *T. comosus* Heuff. are presented in Table 3.

In *Thymus vulgaris* L. extract the identified polyphenolic compounds are: caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin. In *Thymus comosus* Heuff. extract, the identified polyphenolic compounds are: caftaric acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin. The identified compounds in these extracts are the same; except caftaric acid which is present only in *T. comosus* L extract.

Table 2
The parameters of calibration curves

No.	Standard	Rt	slope	intercept
1	Caftaric acid	3.27	25.282	-0.988
3	Caffeic acid	6.10	45.845	-0.981
4	Chlorogenic acid	6.80	26.492	-1.324
5	<i>p</i> -coumaric acid	9.49	33.230	-0.326
6	Ferulic acid	12.80	39.558	-1.017
17	Luteolin	29.93	28.927	-0.761
19	Apigenin	33.95	20.403	-0.909

Table 3
Concentrations of the polyphenolic compounds (mg / 100 g dried vegetal product)

No.	Phenolic compounds	Concentration(mg/100g) <i>T. vulgaris</i> before/ after hydrolysis	Concentration(mg/100g) <i>T. comosus</i> before/ after hydrolysis
1	caftaric acid	-/-	34.8/ 38.4
2	caffeic acid	58.9/ 436.4	147.9/ 432.2
3	chlorogenic acid	27.9/ 25.2	40.7/ 30.9
4	<i>p</i> -coumaric acid	-/ 19.1	-/ 10.9
5	ferulic acid	-/ 41.6	-/ 68.8
6	luteolin	-/ 658.8	42.6/ 107.1
7	apigenin	-/ 57.4	-/ 31.9

After hydrolysis, an increase in caffeic acid concentration was observed, as a result of the hydrolysis of its derivatives (esters). Also, *p*-coumaric acid, ferulic acid, luteolin and apigenin appeared and the luteolin concentration was increased. Apigenin and luteolin are aglycones of flavonoidic O- glycosides that are released by hydrolysis. *T. comosus* L. contains luteolin as free aglycone.

After hydrolysis, a significant decrease in chlorogenic acid and ferulic acid concentrations was observed, as a result of the damage determined by the hydrolysis conditions.

In the chromatogram of hydrolysed extract can be seen some flavonoidic glycosides; they are C-glycosides which can not be hydrolysed in the mentioned conditions.

CONCLUSIONS

The comparative analyses of polyphenolic compounds was made on the vegetal products represented by the aerial parts (*herba*) obtained from two species of *Thymus* (*Thymus vulgaris* L. and *Thymus comosus* Heuff.).

The polyphenolic compounds (flavonoids and phenyl-propane derivatives) were analyzed by spectrophotometric and HPLC methods.

For *Thymus comosus* Heuff., the content of flavonoids is higher than the content in *Thymus vulgaris* L. and the contents of phenyl- propane derivatives are very closed.

Before and after hydrolysis, qualitative and quantitative analyses was performed on caffeic acid, caffeic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin.

The identified compounds in these species are the same; except caffeic acid which are present only in *T. comosus* Heuff. They are in different concentrations.

Both species contain high concentration of caffeic acid.

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