

HPLC-MS ANALYSIS OF THE POLYPHENOLS IN TWO SOFT EXTRACTS OF *ELAEOAGNUS ANGUSTIFOLIA* L. NOTE 2. SOFT EXTRACT OF YOUNG BRANCHES ANALYSIS

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Abstract

In this paper we aimed to establish the content in polyphenolic compounds (flavones and polyphenolcarboxylic acids) in the soft extract of *Elaeagnus angustifolia* L. (oleaster) young branches, using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). The comparative analysis of the chromatograms obtained before and after hydrolysis showed a complex composition. We identified through MS an unknown compound noted as X, which is a rhamnetol or isorhamnetol heteroside because the peak disappears after hydrolysis.

Rezumat

În această lucrare s-a urmărit stabilirea conținutului în compuși polifenolici (flavone și acizi polifenolcarboxilici) a extractului moale din ramuri tinere de *Elaeagnus angustifolia* L., prin cromatografie de lichide de înaltă performanță cuplată cu spectrometria de masă (HPLC-MS). Analiza comparativă a cromatogramelor obținute înainte și după hidroliză a pus în evidență o compoziție complexă. S-a identificat prin MS un compus necunoscut notat X, care este o heterozidă a rhamnetolului sau izorhamnetolului deoarece picul dispare după hidroliză.

Key words: *Elaeagnus angustifolia* L., young branches soft extract, HPLC-MS.

Introduction

Bekker N.P. and all (2001) wrote a review on the researches made between 1950- 2000 on the *Elaeagnaceae* family. They specified in the chemical composition the presence of essence oil, flavones, fats (in flowers) and carotenoids, flavones, tannins, polyphenolcarboxylic acids (in leaves) [1]. The study on the young branches of *Elaeagnus angustifolia* L. (oleaster) indigenous showed that they contain resinic acids, sterols, carotenoids, polyphenols (tannins, flavones) and mucilages [2].

In this paper we aimed to establish the content in polyphenolic compounds (flavones and polyphenolcarboxylic acids) of the soft extract of *Elaeagnus angustifolia* L. young branches. In note 1 of this paper it was established the content in polyphenolic compounds in the flowers soft extract [3].

Materials and methods

To check the presence of the polyphenolic compounds (flavones and polyphenolcarboxylic acids) also in the soft extract of *Elaeagnus angustifolia* L. young branches, the high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) was used.

In this regard we used the soft extract obtained from double maceration with ethanol 40% (2 x 8 days) from young branches powder (100g for 100 mL extractive solution). The extractive solution was submitted to evaporation at 30°C and 0.6 atm. up to soft consistency. Afterwards, the soft extract was hydrolysed with HCl 2N (1:1), for 40 min. on 80°C water bath. The method used was based on a HPLC method published in literature to which some modifications were made [4-8]; the injection volume was 5 µL and the calibration curve was made for each compound between 0.5 – 5 µg/mL concentration intervals. The equipment was a HPLC system coupled with mass spectrometer: HP 1100 series binary pump, autosampler HP 1100 series, thermostate HP 1100 series, UV detector HP 1100 series, mass spectrometer Agilent Ion Trap 1100 VL.

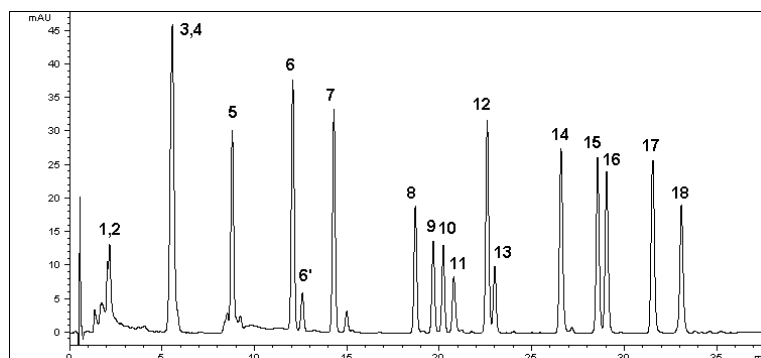


Figure 1

Chromatogram of a mixture of 18 polyphenols, UV detection at 330 and 370 nm

This method [3] can be used for the quality analysis of 18 compounds and the quantity analysis of 14 compounds. There were used 18 standards of polyphenolic compounds: caftaric acid (1), gentianic acid (2), caffeic acid (3), chlorogenic acid (4), p-cumaric acid (5), ferulic acid (6), sinapic acid (7),

hyperoside (8), isoquercitrin (9), rutin (10), myricetol (11), fisetin (12), quercitrin (13), quercetol (14), patuletine (15), luteoline (16), kaempferol (17) and apigenine (18). The chromatogram of a mixture of standards corresponding to polyphenolic compounds mentioned above is given in Fig. 1.

The HPLC method is also used in quantitative determination for the other compounds [9-12].

Results and discussion

The analysis of the UV and MS chromatograms of the unhydrolysed sample of young branches extract identified the following compounds (Table I).

Table I
Polyphenols identified in the unhydrolysed sample of young branches extract

Compounds	No.	Identified UV	Confirmed MS	Observations	Concentration in sample (µg/mL)	Concentration in extract mg/100g extract
p-cumalic acid	5	X	X		94.920	237.30
Ferulic acid	6	X	X		8.440	21.10
Rutin	10	-	X	b, a	-	-
Kaempferol	17	X	X		1.913	4.78

- unidentified, X - identified

a = Qualitative determination based on MS data

b = UV signal too weak for quantization or interferences from other compounds

The UV chromatogram of the unhydrolysed sample of young branches extract is given in Fig. 2.

The MS chromatogram presents the total of all scanned ions. All 104 compounds are numbered. Their mass spectrum was determined by AUTO method.

Through HPLC-UV chromatograms we identified an unknown compound, noted with X and further analyzed through Ms (Fig. 2).

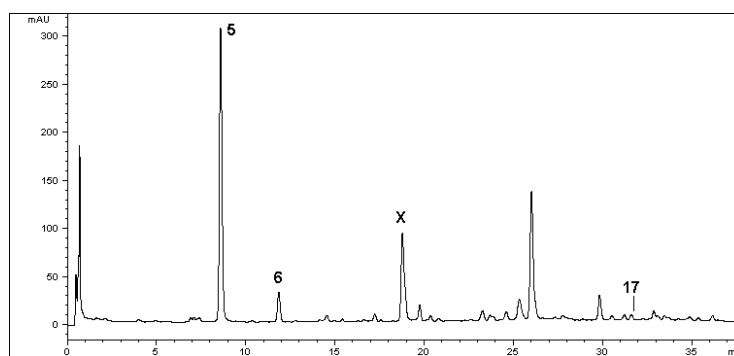


Figure 2

UV chromatogram of the unhydrolysed sample of young branches extract

The X compound elutes in 18-22 min. interval, which is the area of glycoside flavones. Its mass spectrum is given in Fig. 3 and 4.

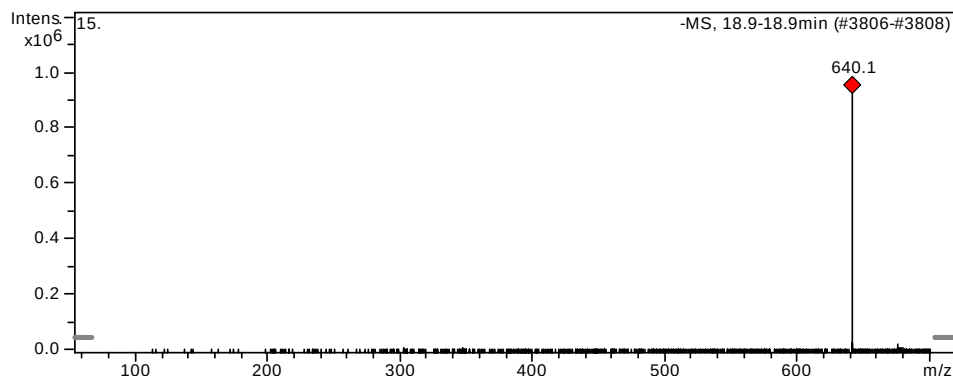


Figure 3

Full-scan mass spectrum of the X compound

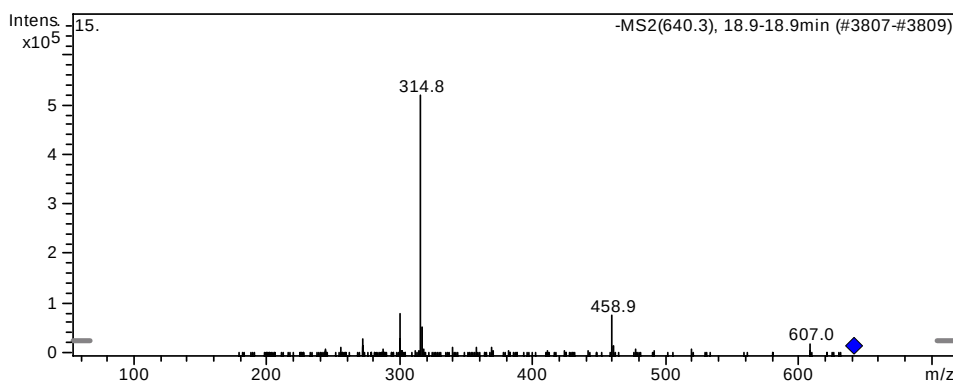


Figure 4

Mass spectrum of the X compound

There can be noticed that the molecular mass is $640 + 1$ (molecule without protons) = 641.

The mass of the agliconic part is $315 + 1$ (proton) = 316, which corresponds to rhamnetol or isorhamnetol. The presence of the rhamnetol heterosides in organs of *Elaeagnus angustifolia* L. is explicable, as these compounds can be also found in *Hippophae rhamnoides* L. species [13] which belong to *Elaeagnaceae* family.

The spectra presented in Fig. 3 and 4 lead us to the conclusion that the X marked peak is a glycoside of rhamnetol or isorhamnetol, with the molecular mass of 641. The saccharide mass can be calculated as $m = 641 - 316 + 18 = 343$. The saccharide can contain two molecules of glucose, galactose, mannose or their combination.

The analysis of the chromatogram of the hydrolysed sample of young branches extract identified the following compounds, given in Table II.

Table II
Polyphenols identified in hydrolysed sample of young branches extract

Compounds	No.	Identified UV	Confirmed MS	Observations	Concentration in sample (µg/ml)	Concentration in extract mg/100g extract
Gentisic acid	2	-	X	a	-	-
Caffeic acid	3	-	X	a	-	-
p-cumaric acid	5	X	X	c	57.909	144.77
Ferulic acid	6	X	X		26.488	66.22
Sinapic acid	7	X	X		4.255	10.63
Quercetol	14	X	X		4.198	10.49
Kaempferol	17	X	X		15.431	38.57

- unidentified, X - identified

a = Qualitative determination based on MS data

c = The compound partially degrades during hydrolysis

The UV chromatogram corresponding to the sample of young branches extract is given in Fig.5.

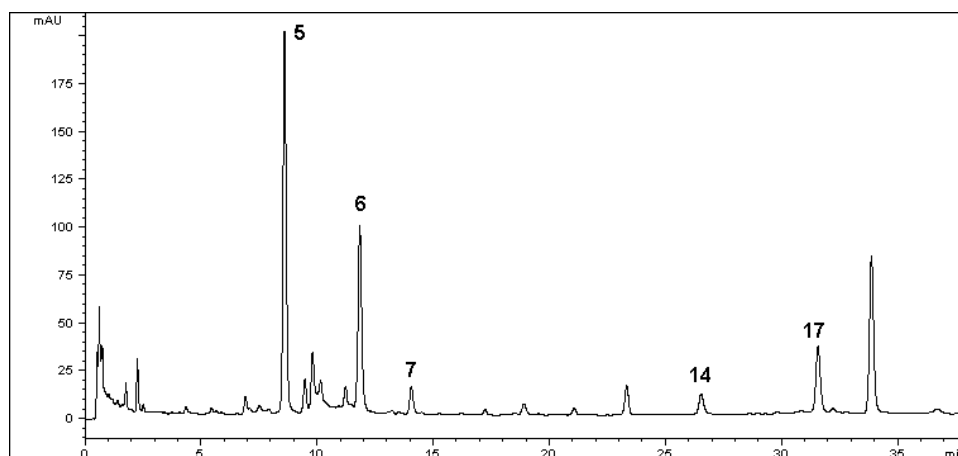


Figure 5

UV chromatogram of the hydrolysed sample with young branches extract

The MS chromatogram presents the total of the scanned ions. All 69 compounds are numbered. Their mass spectrum was determined by AUTO method.

The comparative analysis of the chromatograms obtained before and after hydrolysis showed the following:

- by hydrolysis, rutin (only detected by MS in the unhydrolysed sample) releases only 10.49 mg% quercetol;

- in the hydrolysed sample, the compounds undetected before hydrolysis came out: sinapic acid determined by UV detection (10.63 mg %) and gentisic and caffeic acids only identified by MS;
- 4.78mg% kaempferol before hydrolysis increases to 38.57 mg% after hydrolysis, which can be explained by the existence of this aglicon as a glycoside or ester with the polyphenocarboxylic acids;
- the polyphenolcarboxylic acids identified in both samples (unhydrolysed and hydrolysed) behave differently: in ferulic acid the quantity increases from 21.10 mg% to 66.22mg% while in p-cumaric acid the quantity decreases from 237.30 mg% to 144.77 mg%. This can be explained as follows: in vegetal products the ferulic acid esterifies flavonic aglicons; by hydrolysis, it is freed and its quantity increases. The p-cumaric acid, which can be found in large quantities in the free state degrades during hydrolysis;
- the unknown X compound is a heteroside because the peak disappears after hydrolysis.

Conclusions

Among all the polyphenols identified in the unhydrolysed and hydrolysed samples of soft extract from young branches, two compounds are of great interest: kaempferol, in a larger quantity after hydrolysis (kaempferol heterosides are presented) and p-cumaric acid, in a larger quantity before hydrolysis.

It was also identified an unknown heteroside of rhamnetol or isorhamnetol by MS. Such compounds can be also found in *Hippophae rhamnoides* L. species of the same family, which means that they can be a common element of the species of *Elaeagnaceae* family.

The results of the polyphenolic compounds HPLC-MS analysis from the soft extract studied, justify the research on its antioxidant properties.

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Manuscript received: 01.04.2009