

## Optimization of extraction procedure and determination by high performance liquid chromatography of flavonols and phenolic acids from *Hypericum Perforatum* L.

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### Abstract

**Hypericum perforatum** is a medicinal plant which has been known in traditional medicine as an anti-inflammatory and healing agent. Nowadays the use of *Hypericum* extracts is concerned mainly with antidepressive applications. In the present work, HPLC – RP- C<sub>18</sub> column chromatography with photodiode array detection was applied for the determination of the derivatives of cinnamic and benzoic acid (e.g., caffeic, chlorogenic, ferulic, sinapic, gallic acids) (Fig.1.) and flavonols - quercetine derivatives (quercetine, rhamnetine, quercitrin, mirycetine, keampferol and rutin) in *Hypericum Perforatum*. Phenolic compounds were extracted from the sample matrix with ethanol and ethanol-water mixture in different ratios solvent (3:7; 8:2; v/v) at 30°C and 60°C in water-bath shaker and by ultrasonic extraction and then analyzed before and after acid and basic hydrolysis. The total amount of studied flavonols and phenolic acids were compared with the total flavonoids content (TFC) and with total polyphenols content (TPC). UV-Vis spectrometry was used to investigate methods for qualitative and quantitative determination of these compounds.

**Keywords:** *Hypericum perforatum* L., HPLC-PDA, flavonols, phenolic acids, TPC, TFC

### 1. Introduction

*Hypericum perforatum* L. is a herbaceous perennial plant widely distributed in Europe, Asia, and North Africa; it is also naturalized in North America [1]. It is well known as “St. John’s wort” in Anglo-Saxon folk medicine and “Erba di S. Giovanni” in Italy. It is well known as a medicinal plant and its extracts are used as an anti-inflammatory, healing agent and an antidepressant medicine, which has been demonstrated in numerous clinical trials challenging the conventional

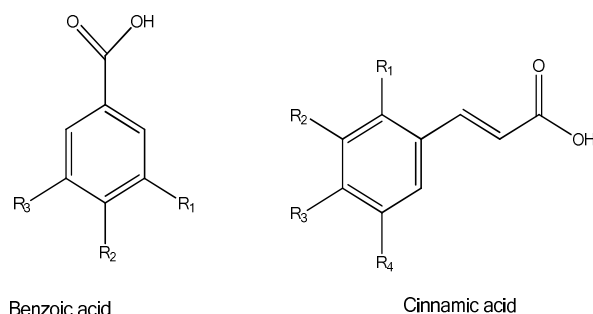
antidepressant drugs [2]. The part of this activity is attributed also to the drug antimicrobial activity [3]. *Hypericum* extracts are marketed not only as herbal medicinal products but also in the form of dietary supplements [4]. In recent years, the consumption of *H. perforatum* - derived products has increased dramatically and presently it is one of the most consumed medicinal plant over the world [5].

*Hypericum perforatum* contains a variety of constituents with documented biological activity including naphthodianthrones (hypericin, pseudohypericin, protohypericin and protopseudohypericin), phloroglucinols (hyperforin and adhyperforin), a broad range of flavonoids (quercetin, quercitrin, isoquercitrin, hyperoside, astilbin, miquelianin, I3 and I18-biapigenin), and phenolic acids (chlorogenic acid and 3-O-coumaroylquinic acid), epigallocatechin, and other minor components. [3] The flavonoids comprising the aglycone quercetin and several glycosides, namely hyperoside (quercetin-3-galactopyranoside), rutin (quercetin-3-rhamnosylglucoside) and quercitrin (quercetin-3-rhamonopyranoside). Flavonoids present in *H. perforatum* L. extracts have been shown to have antidepressive activities [6].

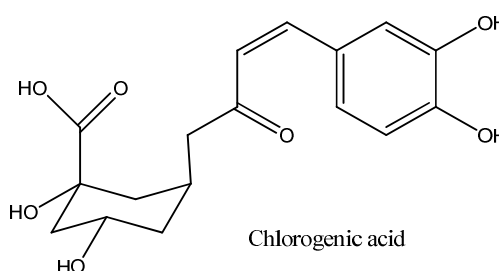
Determination of compounds in plant materials requires extraction from the sample matrix prior to injection into the HPLC system. Flavonoids are commonly extracted from herbal samples with pure

methanol, ethanol or their combination with water [7-9], but in some cases ethyl acetate [4] or acetone [10] have been used. The optimum extraction conditions varied depending on the active compounds as well as on the kind of plant. Determination of individual flavonoid glycosides in plant materials is difficult, due to their large number. In many cases the knowledge of the total aglycone content for each flavonoid is required. Walle et al. [11] reported, that the dietary form of flavonoids, the flavonoid glycosides, may be hydrolyzed in the oral cavity by bacteria to deliver the biologically active aglycones at the surface of the epithelial cells. Hydrolysis of all glycosides to aglycones allows to obtain more accurate data on flavonol concentrations in food samples. This process is dependent on the concentration of HCl, hydrolysis time and temperature and the composition of the extraction solvent [12]. Moreover, different extraction procedures are indicated in the various Pharmacopeias.

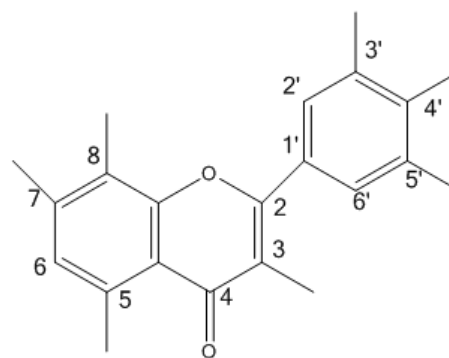
Isolation and purification of bioactive compounds in *H. perforatum* L. using HPLC and macroporous resin has been reported [13-15]. Generally, HPLC methods of *H. perforatum* preparations are concerned with the determination of flavonoids content [16]. High-speed counter-current chromatography (HSCCC), being a support-free liquid-liquid partition method [17], eliminates irreversible adsorption of sample onto the solid support, and has been widely used in preparative separation of natural products [18-20]. CCC has also been used in isolating flavanoids from *H. perforatum* L. [22]. In many cases, preparative high-performance liquid chromatography (prep-HPLC) is a method needed to satisfy the purity specifications required on a routine basis, and it is also an important industrially applied separation process for the isolation and purification of pharmaceuticals and other valuable products [22]



Benzoic acid :  
Gallic acid (R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=OH)  
Cinnamic acid:  
Ferulic acid (R<sub>1</sub>=R<sub>2</sub>=H; R<sub>3</sub>=OH; R<sub>4</sub>=OCH<sub>3</sub>);  
Caffeic acid (R<sub>1</sub>=R<sub>4</sub>=H; R<sub>2</sub>=R<sub>3</sub>=OH);  
Sinapic acid (R<sub>1</sub>=H; R<sub>3</sub>=OH; R<sub>2</sub>=R<sub>4</sub>=OCH<sub>3</sub>);



**Fig. 1.** Structural skeletons of main phenolic acids [23]



Quercetin 3, 5, 7, 3', 4' = OH  
Mirycetin: 3, 5, 7, 3', 4', 5' = OH  
Kaempferol: 3, 5, 7, 4' = OH

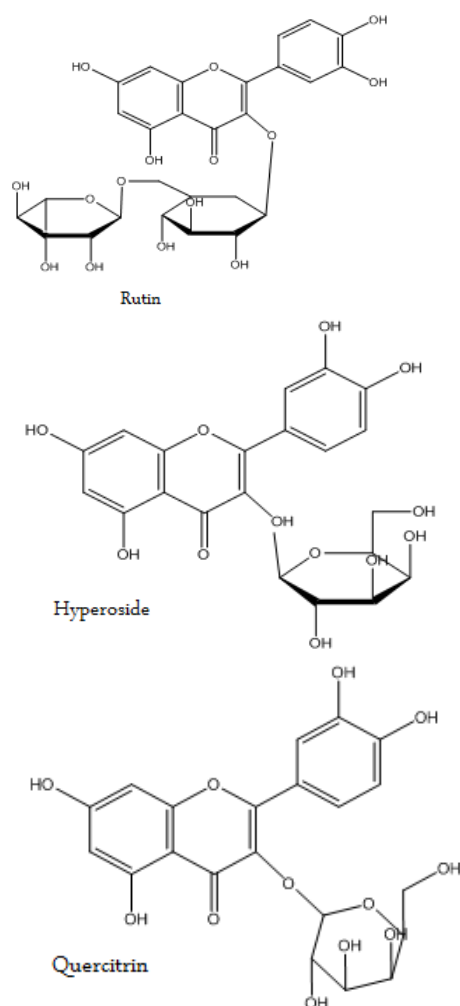


Fig. 2. Structural skeletons of main flavonols [23]

The main aim of this work was to investigate the optimum conditions for efficient extraction of studied flavonols and benzoic and cinamic acid derivatives from *H. perforatum*. For quercetin analysis as aglycone the effects of acid concentration and hydrolysis time on the extraction recovery were also investigated.

## 2. Experimental

### 2.1. Chemicals and samples

Every one of quercetin derivatives and also caffeic and chlorogenic acids were purchased from SIGMA ALDRICH (Germany), while ferulic, sinapic acids from FLUKA (USA). Gallic and benzoic acids were purchased from POCh (Gliwice, Poland).

The phenolic compounds were isolated from *Hypericum Perforatum* L. (purchased from the Pharmaceutical Company, Biofaktor, Gorzów Wielkopolski). The purity and structural identity of each isolated compound were chemically characterized by HPLC-PDA.

Methanol CHROMASOLV®, isopropanol and tetrahydrofuran were from SIGMA Aldrich (Germany). Every one of solvents were HPLC grade. Methanol, ethanol, glacial acetic acid, hydrochloric acid (35-38%) and sodium hydroxide were from Chempur (Piekary Śląskie, Poland). Folin-Ciocalteu's Phenol Reagent and disodium carbonate were purchased from POCh (Gliwice, Poland).

### 2.2. Apparatus

The HPLC system consisted of liquid chromatograph equipped with auto sampler SIL-20AC HT a photodiode multi-wavelength detector (SPD-M20A prominence diode array detector) made by SHIMADZU (Kyoto, Japan). The absorption was measured either as a full spectrum (200–800 nm) and at 254 nm (for benzoic and gallic acids), 325 nm (for caffeic, chlorogenic, sinapic and ferulic acids) and 360 nm for quercetin derivatives. The chromatographic data were recorded and processed by the *LCsolution version 1.23 SP*.

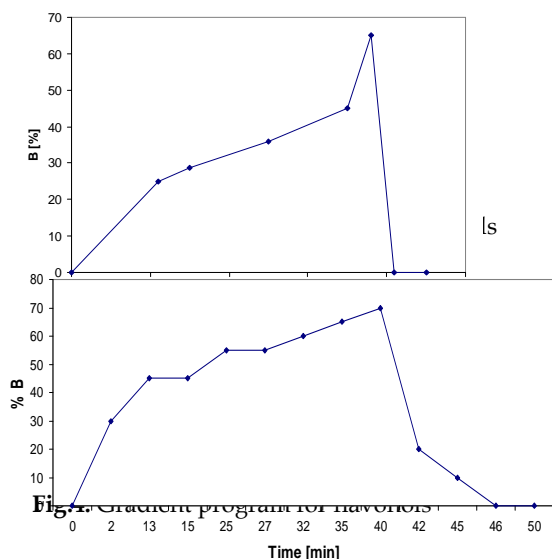
UV-VIS spectra were recorded on a Spectrophotometer UV Unikam HELIOS  $\alpha$ , manufactured by Spectro-Lab (Warsaw, Poland). 1 cm quartz cuvette was using.

### 2.3. Chromatographic conditions

The chromatographic data were recorded and processed by the *LCsolution version 1.23 SP*. Analyses were carried out at on Discovery RP-C<sub>18</sub> column (5 µm particle size, 150×4,6 mm, SUPELCO). A column was maintained at 30°C.

For phenolic acids mobile phase A was 2% acetic acid and phase B was methanol (HPLC grade). For studied flavonols, mobile phase A was mixture of water-isopropanol (95:5, v/v) and phase B were water:isopropanol:THF (50:40:10, v/v). The analysis every one of studied polyphenols followed a nonlinear gradient program (Fig. 3 and Fig.4).

The flow-rate was kept constant at 1 ml/min. The injection volume was 10 µl and 20 µl for phenolic acids and flavonols, respectively. The detection was performed at the maximum UV-VIS absorptions of the ten compounds: at 254 nm (for benzoic and gallic acids) and 325 nm (for caffeic, chlorogenic, sinapic and ferulic acids) and 360nm for every one of studied flavonols.



### 2.4. Calibration curves

Gallic, caffeic, chlorogenic, ferulic, sinapic, benzoic acids were dissolved in methanol/water mixture (8:2, v/v), while flavonols were dissolved in MeOH (HPLC grade) and diluted to appropriate concentration ranges for the construction of

calibration curves. The calibration curves were based on the analysis of 5 repetition standards at 12 concentration levels for phenolic acids (Table 1) and for quercetin derivatives (Table 2). These curves were constructed by plotting the area peak as a function of the concentration.

### 2.5. Extraction procedure

The extraction system consisted of ethanol (EtOH) and ethanol-water in different ratios solvent (3:7; 8:2; v/v) for phenolic compounds. The total volume of cold extract was shared for 3 portions (at the same volume). One of these extract portions was filtered through a soft quantitative filter paper into a chromatographic vial and volumetric flask.

Two portions of the extract were successively hydrolyzed by acidic and basic digestion. Samples were analyzed before and after acidic and basic hydrolysis.

If the extract was not clear, about 2 ml of the extract was transferred to 5 ml centrifuge tubes and centrifuged for 15 min at high speed centrifuge. The clear supernatant was transferred to an LC sample vial. Extractions were performed in duplicate. The detail procedures for HPLC quantitative analysis were described above.

### 2.6. Quantification of polyphenols in *Hypericum perforatum* L.

0.5 gram (0.01g) of fine powder *Hypericum perforatum* L. were extracted with ethanol and ethanol-water in different ratios (3:7; 8:2; v/v) solvents two times. Each extraction was performed using a water - bath shaker at 30°C and 60°C within 1 and 2 hour and also using ultrasonic extraction at the same temperature within 15 and 30 min.

### 2.7. Extraction followed by acid or/and basic hydrolysis

Three portions of the extract were successively hydrolyzed by acidic and basic

digestion. Samples were analyzed before and after acidic and basic hydrolysis. The solution was filtered through a soft quantitative filter paper or centrifuged before HPLC analysis.

**Basic hydrolysis** (was assigned II): Five milliliters of the extract was placed in a 100 ml conical flask and 300 µl of 3 M NaOH was added. The extract was swirled at 30°C for 30 min.

**Acid hydrolysis** (was assigned III): Five milliliters of extract was placed in a 100 ml conical flask with 1 ml of concentrated HCl. The flasks were heated for 30 min at 30°C in a water-bath shaker.

## 2.8. UV-VIS spectral studies

### 2.8.1. The total polyphenols content (TPC)

The amount of total polyphenols in the extract was determined according to the Folin-Ciocalteu method. Caffeic acid and quercetin were employed as a calibration standard and results were expressed as caffeic acid and quercetin equivalents, respectively. The absorbance of the test solution was measured at 725 nm after 1 h.

Reagents were dissolved in bidistilled water. For quantitative assays, the solution were prepared by appropriate dilution (1.0; 2.0; 4.0; 6.0; 8.0; 10.0; 12.0; 16.0 µg/ml) of freshly prepared stock-solution (20µg/ml). Before diluting, 0.20 ml Follin-Ciocalteu's solution and 0.50 ml of disodium carbonate solution (0.07g/ml) were added. The calibration curves were based on the analysis of 5 repetition standards at 8 concentration levels. These curves were constructed by plotting the absorbance as a function of the caffeic acid concentration.

Test solution - 0.60 ml Folin-Ciocalteu's Phenol Reagent and 0.50 ml of disodium carbonate solution (0.07g/ml) were mixed in 10 ml volumetric flask and diluted with distilled water. Test solution was kept without light for 1 h. For sample assaying, 50 µl of extract was introduced to 5 ml volumetric flask.

### 2.8.2. The total flavonoids content (TFC)

The total flavonoids content (TFC) was determined using a colorimetric method described in Polish Farmacopoeia. Quercetin was employed as a calibration standard and results were expressed as quercetin equivalents. The absorbance of the test solution was measured at 428 nm after 30 min. [24]

Quercetin was dissolved in ethanol. For quantitative assays, the solution were prepared by appropriate dilution (1.0; 3.0; 5.0; 7.0; 9.0; 10.0; 12.0; 14.0 µg/ml) of freshly prepared stock-solution (20 µg/ml). Before diluting in mixture of acetic acid (1,02 kg/l) and methanol (1:19, v/v), 1.20 ml aluminium chloride (20 g/l) solution was added. The calibration curves were based on the analysis of 5 repetition standards at 8 concentration levels. These curves were constructed by plotting the absorbance as a function of the quercetin concentration.

Test solution -1.20 ml aluminium chloride (20 g/l) were put in 10 ml volumetric flask and diluted with mixture of acetic acid (1,02 kg/l) and methanol (1:19, v/v). Test solution was kept without light for 30 min. For sample assaying, 50 µl of extract was introduced to 10 ml volumetric flask.

## 3. Result and discussion

Peaks on the chromatograms were identified by comparison of the retention times and UV spectra of the peaks in extractions with those of the reference standards. Peaks were also identified by addition of the individual reference standard to extracts. The purity of each peak was checked by PDA software. Typical chromatograms for *Hypericum perforatum* extract are presented at Fig. 5. Because of complex biological samples, identification every peaks was impossible. Gallic and benzoic acids were detected at

254 nm, while chlorogenic, caffeic, ferulic, sinapic acids at 325 nm. Derivatives of quercetine (quercetine, rutin, quercitrin, rhamnetin, mirecetine, kaempferol and hyperoside) were detected at 360 nm.

The regression coefficient of each calibration curve was higher than 0.99. Calibration curves in HPLC-PDA method were constructed by plotting the area peak as a function of the concentration. The results are collected in Table 1 and Table 2. Detection limit (DL) was not lower than 0.28 mg/l and 0.35 mg/l (for hyperoside and gallic acid, respectively) and not higher than 1.82 mg/l and 7.55 mg/l (for quercitrin and chlorogenic acid, respectively) and range of quantification limit (QL) was 1.08 – 24.04 mg/l (Table 1) and 0.85 – 5.51 mg/l (Table 2).

In spectrophotometry method – TPC and TFC - calibration curves were constructed by plotting the absorbance as a function of the concentration of the caffeic acid and quercetine, respectively. The regression coefficient of each calibration curve was also higher than 0.99 (unpublished data). Detection limit (DL) for TFC and TPC was 0.31 µg/ml and 0.63 µg/ml, respectively.

All the examined extracts were significantly active as antioxidant agents. The result of the total polyphenols amount (TPC) in the extracts determining by the Folin-Ciocalteu method are presented at Figure 6, including the impact of various extraction conditions, such as method (water+bath and ultrasonic extraction), time (15 and 30 min, and also 1 and 2 h), and temperature (30°C and 60°C). The best results were obtained by ethanolic extraction. Obtained results demonstrate also, that not only solvents have influence for extraction proces. The highest concentration of polyphenolic compounds were obtained by ultrasonic extraction at 60°C and 30 min. In every cases ultrasonic extraction increase recovery and short preparation time.

At Figure 7 are presented correlation between total phenolic content (TPC) and total flavonoids content (TFC). The correlation is good (the regression coefficient  $R^2=0.9758$ ). In every cases, total concentration of polyphenols (TPC) was higher than total amount of flavonoids and flavonols determination by HPLC-PDA method (presented in Table 3). TFC was also higher than concentration of studied phenolic acids and studied flavonols determination by HPLC-PDA method. In general flavonols (such as rutin, quercetin, quercitrin kaempferol and hyperoside) are among the major compounds in *Hypericum perforatum* herb. However studied flavonols and phenolic acids constitute only smart part of total polyphenols presented in *H. perforatum* L.

At Figure 8. are presented impact of different extraction conditions (only ultrasonic extraction) and hydrolysis digestion for the total phenolic content (TPC) in *Hypericum perforatum* herb. Hydrolysis step has been included to minimize interferences in subsequent chromatography and to simplify chromatographic data. Acidic and alkaline hydrolysis, prior to extraction caused the increase the quantitative yield. The influence of hydrolyzed digestion is significantly. For determination polyphenols the highest concentration were obtained after acidic hydrolysis. Acidic hydrolysis was better for flavonols and phenolic acids, while alkaline hydrolysis was adequate for phenolic acids. Acidic hydrolysis has been used to measurement of aglycones and phenolic acids from flavonoid glycosides and phenolic acid esters.

Alkaline hydrolysis has been approached in the isolation of phenolic acids, due to they commonly form covalent bond with the sample matrix. Phenolic acids exist in a wide range of conjugated forms.

At Table 4 there were presented literature data for comparison of flavonols

content [25-28]. The range of concentration of studied quercetine derivatives determination by HLC-PDA method before and after hydrolysis digestion were the same at demonstrated data. We could

compare our data with others authors data and corroborate that describing in this paper HPLC-PDA method is precision, selective and accuracy.

Table 1.

Linear calibration curves for six phenolic acids (standards)

Phenolic acid	y = ax +b, *the linear model		Regression coefficient r <sup>2</sup>	Range of concentration [mg/l]	DL [mg/l]	QL [mg/l]
	a±S <sub>a</sub>	b±S <sub>b</sub>				
gallic acid	18480±188	5412±1186	0.9990	0.06 – 130	0.35	1.08
chlorogenic acid	28406±264	136259±69405	0.9992	0.05 - 600	7.98	24.04
caffeic acid	54327±430	48342±48260	0.9994	0.10 – 300	7.55	22.87
ferulic acid	52874±292	11271±29473	0.9997	0.50 - 200	4.63	14.04
sinapic acid	52692±815	78950±27412	0.9976	0.10 - 70	3.30	10.00
benzoic acid	5024±41.7	18527±4387	0.9994	0.50 – 200	6.66	20.19

\*x- concentration, y- peak area; a – slope; b – intercept, ±S<sub>a</sub>, ±S<sub>b</sub> – standard deviation of slope and intercept; detection limit DL=(3.3S<sub>x/y</sub>)/a; quantification limit QL=(10S<sub>x/y</sub>)/a; S<sub>x/y</sub> – standard deviation of y-residuals

Table 2.

Linear calibration curves for quercetin derivatives (standards)

Flavonols	y = ax +b, *the linear model		Regression coefficient r <sup>2</sup>	Range of concentration [mg/l]	DL [mg/l]	QL [mg/l]
	a±S <sub>a</sub>	b±S <sub>b</sub>				
quercetin	30065±412	5131,3±9739	0.9987	2.24 – 44.80	1.80	5.47
rutin	22164±261	5104,3±7324	0.9990	4.16 – 52.00	1.74	5.27
hiperoside	16979±1529	- 33514±7802	0.9994	0.40 – 10.00	0.28	0.85
kaempferol	37672±330	- 18017±2529	0.9994	0.60 – 15.00	0.41	1.24
rhamnetin	24676±651	- 3452,8±3322	0.9945	0.40 – 10.00	0.82	2.49
quercitrin	44009±734	- 80465±13117	0.9978	1.40 – 35.00	1.82	5.51
mirecetine	21217±449	- 11759±2638	0.9978	0.80 – 10.00	0.58	1.75

\*x- concentration, y- peak area; a – slope; b – intercept, ±S<sub>a</sub>, ±S<sub>b</sub> – standard deviation of slope and intercept; detection limit DL=(3.3S<sub>x/y</sub>)/a; quantification limit QL=(10S<sub>x/y</sub>)/a; S<sub>x/y</sub> – standard deviation of y-residuals

Table 3.

Quercetine derivatives content of *Hypericum perforatum* L. extract determined by HPLC-PDA method (ultrasonic extraction)

Name of flavonols	Quercetine derivatives content of <i>Hypericum perforatum</i> extract (ultrasonic extraction) [mg/g s.m ± SD]			
	15min		30min	
	30°C	60°C	30°C	60°C
Rutin	1.56 ± 0.01	2.13 ± 0.03	2.73 ± 0.03	3.41 ± 0.13
Hyperoside	0.61 ± 0.001	0.89 ± 0.01	1.00 ± 0.02	2.40 ± 0.06
Quercitrin	1.04 ± 0.002	1.40 ± 0.01	2.52 ± 0.01	3.85 ± 0.02
Mirecetine	0.43 ± 0.017	0.54 ± 0.004	0.69 ± 0.002	0.97 ± 0.02
Quercetine	0.47 ± 0.004	1.57 ± 0.009	2.71 ± 0.02	4.93 ± 0.014
Keampferol	0.39 ± 0.005	0.52 ± 0.004	0.7 ± 0.0005	0.94 ± 0.01
Rhamnetine	Nd	0.003 ± 0.0001	nd	0.10 ± 0.002
<b>TOTAL</b>	<b>4.53 ± 0.02</b>	<b>7.05 ± 0.04</b>	<b>10.37 ± 0.03</b>	<b>14.63 ± 0.14</b>

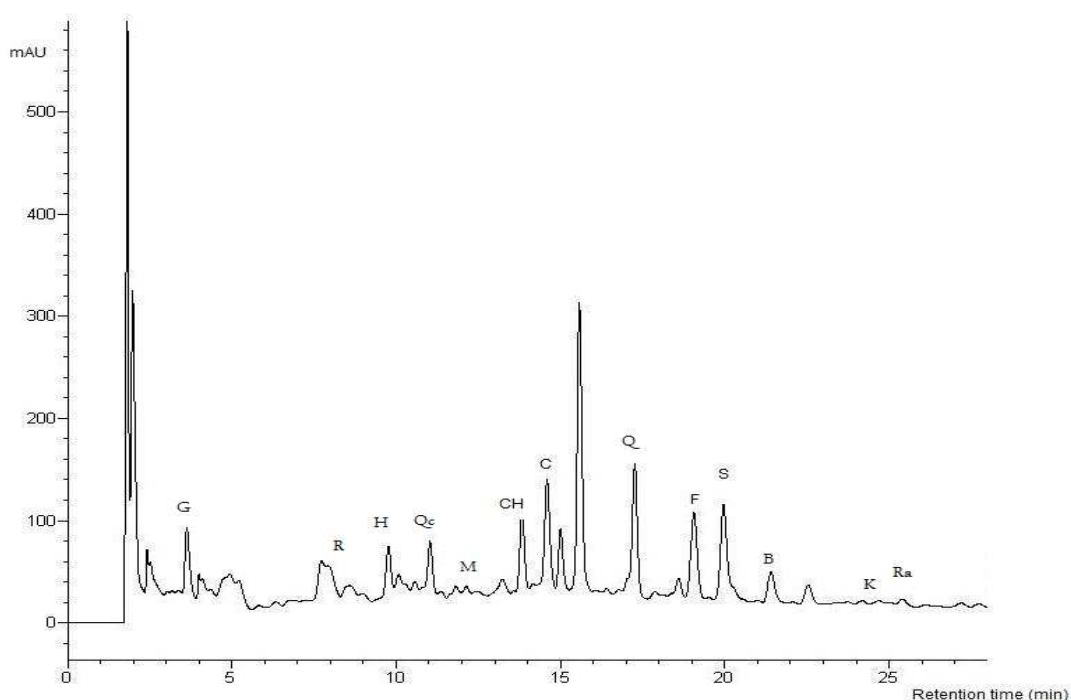


Fig.5. HPLC chromatograms at 254, 325 and 360 nm for *Hypericum perforatum* L. extract. G-gallic acid, R-rutin, H-hyperoside, Qc-quercitrin, M-mirecetine, CH-chlorogenic acid, C-caffeic acid, Q-quercetin, F-ferulic acid, S-sinapic acid, B-benzoic acid, K-keampferol, Ra-rhamnetin.



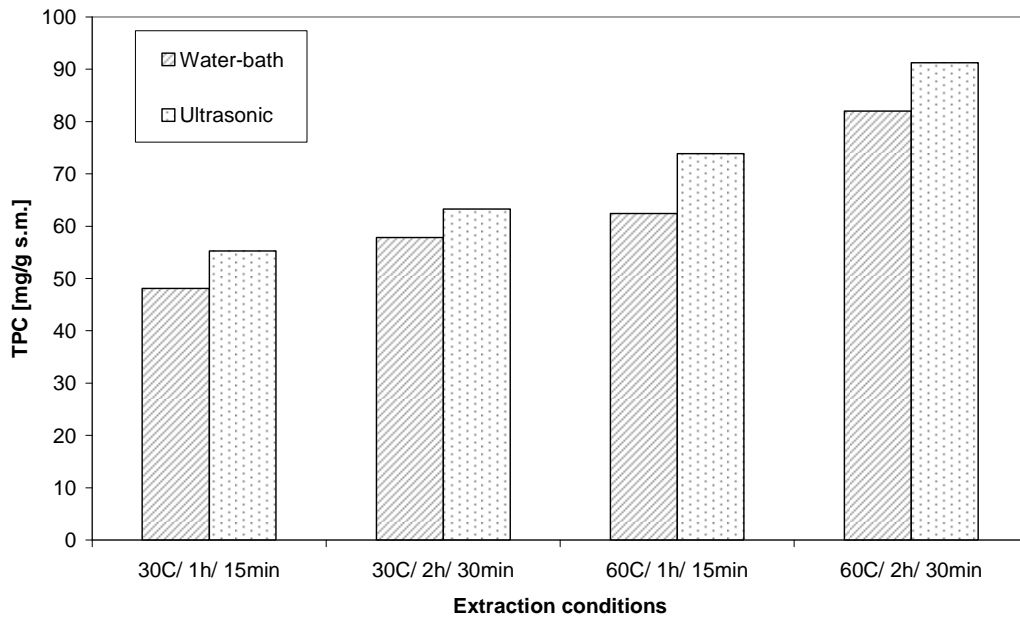


Fig. 6. Impact of various extraction conditions (method, time and temperature) for the total phenolic content in *Hypericum perforatum* L.

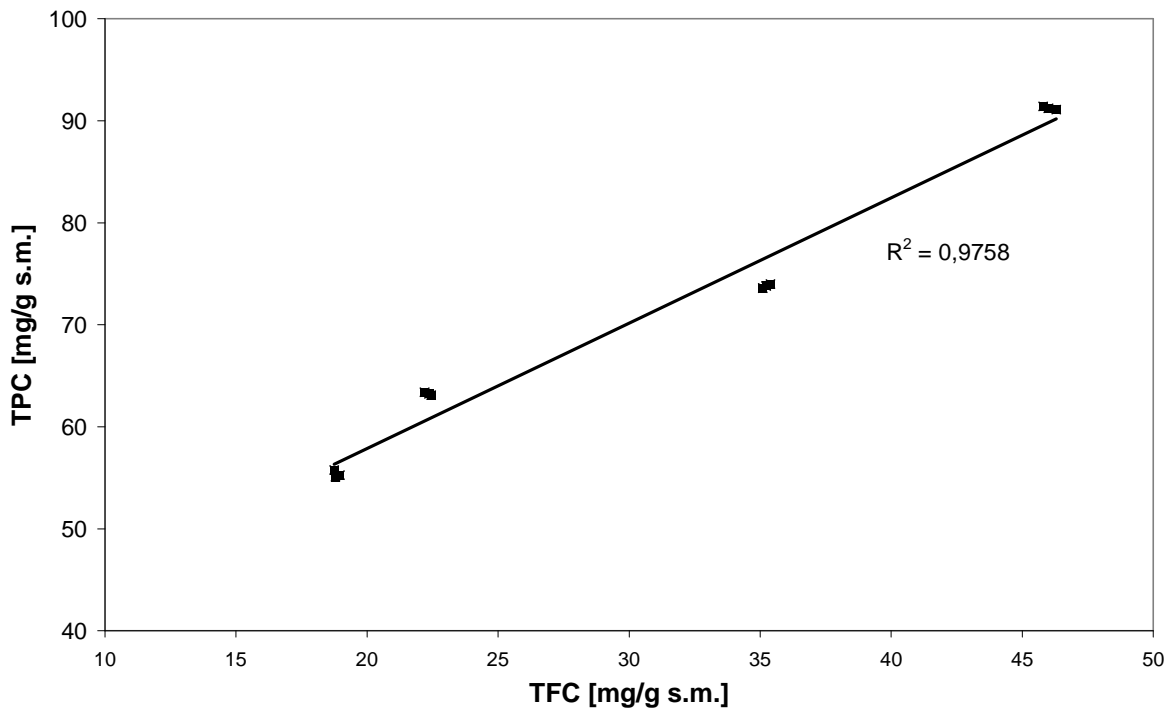


Fig. 7. Correlation between TPC and TFC

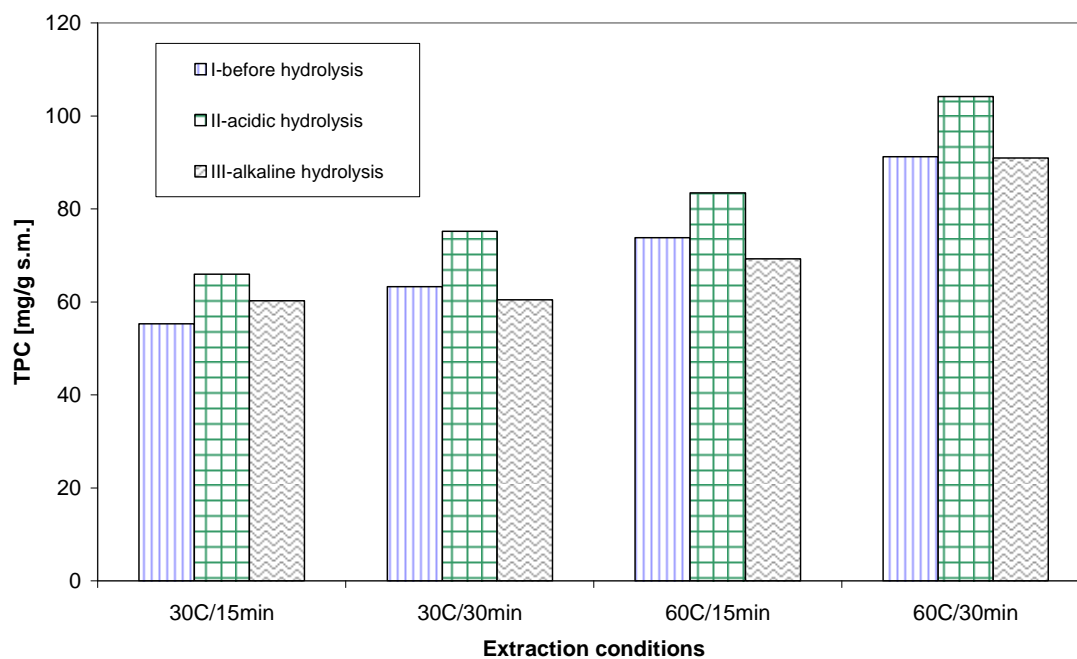


Fig. 8. Impact of different extraction conditions and hydrolysis digestion for the total phenolic content in *Hypericum perforatum* L.

Table 4.

Comparison of flavonols content – optimization

Flavonols content [mg/g s.m. ± SD]	[25]	[26]		[27]	[28]	HPLC			
	E	leaves	Flowers	E	E (Hkw)	E	H (kw)		
		E	H(kw)	E	H(kw)				
<b>Quercetine</b>	0.9 – 2.5	0.31 ±0.02	6.94 ±0.34	7.52 ±0.36	12.4 ±0.62	12.6	0.26-1.54	0.47-5.93	3.98- 11.23
<b>Rutin</b>	6.7 – 23.1	3.45 ±0.16	0.54 ±0.03	1.49 ±0.06	0.61 ±0.03	---	6.54- 23.30	1.56-3.41	0.71-1.10
<b>Quercitrin</b>	0.6 – 2.5	0.38 ±0.02	nd	1.84 ±0.09	0.71 ±0.03	7.8	1.33- 12.85	1.04-3.85	0.57-0.83
<b>Hyperoside</b>	3.7 – 15.8	1.78 ±0.09	nd	4.70 ±0.23	0.52 ±0.02	---	13.74- 41.64	1.04-5.49	0.15-0.78
<b>TFC (UV-Vis)</b>	---	<b>31.70</b> <b>±0.33</b>	<b>44.70</b> <b>±0.21</b>	<b>42.91</b> <b>±0.25</b>	<b>65.25</b> <b>±0.18</b>	<b>54.3</b>	<b>208.4- 271.1</b>	<b>18.97- 46.30</b>	<b>26.13- 61.80</b>

Nd – not detected; E-extraction, H(kw)-acidic hydrolysis, TFC- total flavonoids content

#### 4. Conclusion

In conclusion, water-bath and ultrasonic extraction method with different extraction conditions (temperatures, solvents) and influence of hydrolysis digestion was evaluated. A RP-HPLC-PDA method was developed for quantify simultaneously six phenolic acids and seven flavonols, which are biologically active components in *Hypericum Perforatum* L. In most cases the highest concentrations of studied phenolic compounds were obtained for ultrasonic extraction by ethanolic solvents at 30 min and 60°C. Depending of extraction conditions (extraction method, solvents, time, temperature, acidic or alkaline hydrolysis) we received different amounts of total phenolic compounds, total flavonoids content and studied flavonols determination by HPLC-PDA method (the average values were 104.18±1.14; 61.80±0.53; 14.63±0.44 mg/g respectively). Hydrolyzed digestions have also significantly impact for polyphenol's quantity. Acidic and alkaline hydrolysis, prior to extraction caused the increase the quantitative yield. Total content of studied compounds were compared with total phenolic content (TPC) and total flavonoids content (TFC) determination using spectrophotometric method. In every cases phenolic acids and flavonols constitute only small amount of total polyphenols. while concentration of flavonols was higher than phenolic acids. Because of positive impact of human health and bioavailability of flavonols, flavonoids and phenolic acids in botanical products and natural plant sources, their qualitative and quantitative analysis are necessary and justified.

In this paper, describing HPLC-PDA method may be applicability to routine analysis and quality control of pharmaceutical products from *Hypericum perforatum* L., because of its simplicity, easy and fast preparing sample and short analysis time. Because of wide range of extraction conditions, analysing a lot of

botanical samples we may ascertain that obtained results and developed method are reliable and dependable. The proposed method is precision, selective and accuracy.

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