PHYTOCHEMICAL INVESTIGATION OF *CRATAEGI FOLIUM CUM FLOS* (HAWTHORN LEAVES AND FLOWERS) AND *HYPERICI HERBA* (ST JOHN`S WORT AERIAL PARTS) HYDROALCOHOLIC EXTRACTS

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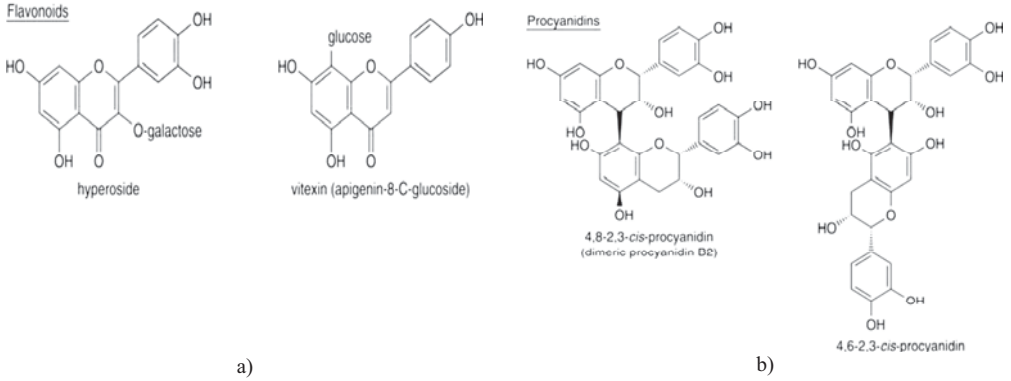
Keywords. Hawthorn, St John`s Wort, flavonoids, polyphenols.
Abstract. Hawthorn (*Crataegus monogyna*) hydroalcoholic extract was prepared by extraction of powdered dried leaves and flowers with ethanol 70% v/v (1:10), by reflux for two hours. St John`s Wort (*Hypericum perforatum*) hydroalcoholic extract was prepared by extraction of powdered dried flowering aerial parts with ethanol 70% v/v (1:10), by reflux for two hours. Both extracts were qualitative and quantitative analyzed. The qualitative analysis consisted in phytochemical screening and spectroanalytical profile by HPTLC and UV/VIS absorption spectroscopy. Quantitative analyses consisted in determination of total flavonoid content (as rutin) by following colorimetric aluminum chloride method, and total polyphenol content (as gallic acid) by Folin- Ciocalteu method. Results were evaluated statistically and presented as mean of three determinations ± SD (standard deviation). These analyses revealed their complex composition and either some similarities and differences between these two extracts.

INTRODUCTION

Several species of the genus *Crataegus* L. (hawthorn) have been reported to possess a wide range of pharmacological actions on the cardiovascular system. Preparations of hawthorn including leaf, flower and berry, have been used traditionally in minor forms of coronary heart disease, heart failure and cardiac arrhythmia, but only approved monograph by German Commission E is hawthorn leaf with flower with the unique indication of decreasing cardiac output, classified by the New York Heart Association (NYHA) as stage II. Hawthorn leaf with flower consists of dried flowering tops of *C. monogyna* Jacq. or *C. laevigata* (Poir.) DC. (syn. *C. oxyacantha* L.) (Amanzadeh *et al*., 2007). Hawthorn is known for its polyphenolics among which the major active constituents are flavonoids including hyperoside and rutin as flavonol – O -glycosides, vitexin, vitexin-2``- O - rhamnoside and acetylvitexin - 2``- O - rhamnoside as flavone – C – glycosides (Fig.1a); proanthocyanidins including oligomeric procyanidins (Fig.1b); phenolic carboxylic acids including caffeic acid and chlorogenic acid (Amanzadeh *et al*., 2007; Barnes *et al*., 2007; Braun and Cohen, 2007; Wagner and Bladt, 2009; Wagner *et al*., 1984).



a) b)

Fig. 1. Structures of flavonoids (a) and procyanidins (b) (Barnes *et al*., 2007)

St John’s Wort (*Hypericum perforatum* L.) has a chemical composition well studied: anthraquinone derivatives (naphthodianthrones) - hypericin, pseudohypericin (Fig. 2b) and isohypericin, protohypericin and protopseudohypericin (biosynthetic precursors of hypericin and pseudohypericin, respectively); flavonoids - flavonols (kaempferol, quercetin), flavones (luteolin) and glycosides (hyperoside (Fig. 2a), isoquercitrin, quercitrin, and rutin),

biflavonoids including biapigenin (a flavone) and amentoflavone (a biapigenin derivative) and catechins (flavonoids often associated with condensed tannins; prenylated phloroglucinols (Fig. 2c)- hyperforin and adhyperforin; tannins; procyanidins (condensed type); other phenols- caffeic, chlorogenic, p-coumaric, ferulic, p-hydroxybenzoic and vanillic acids; volatile oils, carotenoids, choline, β- sitosterol etc. It is stated to possess sedative and astringent properties, cytotoxic and anticancer, antimicrobial and antiviral activities (Barnes *et al*., 2007; Braun and Cohen**,** 2007; Duke, 2002; Wagner and Bladt, 2009; Wagner *et al*., 1984).

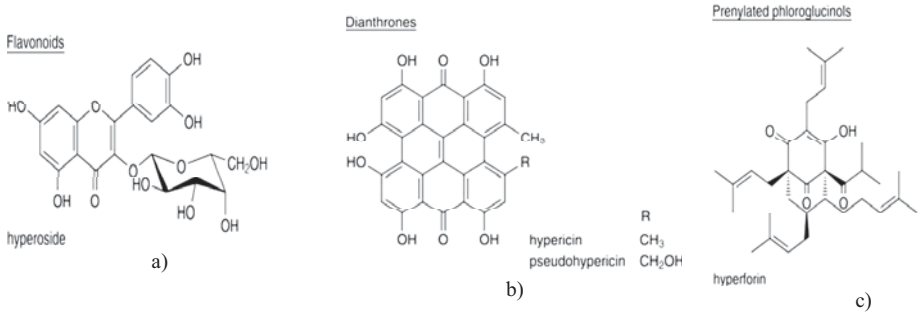


Fig.2. Structures of flavonoids (a), dianthrones (b) and prenylated phloroglucinols (c) (Barnes *et al*., 2007)

The objective of this study was the qualitative and quantitative analysis of the active principles in the 70% hydroalcoholic extracts of hawthorn (*Crataegi folium cum flos*) and St John's wort (*Hyperici herba*).

MATERIALS AND METHODS

Chemicals. Rutin, hyperoside, caffeic acid and 2, 2 – diphenyl -1 – picrylhydrazyl (DPPH) were purchased from Sigma – Aldrich Co., gallic acid (Fluka) from Sigma – Aldrich Chemie GmbH, chlorogenic acid from Fluka Chemie, Sigma – Aldrich, and Folin-Ciocalteu reagent was purchased from Merck KGaA. All other chemicals were of analytical grade or pure.

Extracts preparation. The fluid hydroalcoholic extract of *Crataegus monogyna* (hawthorn) leaves and flowers (CMEx) was obtained from powdered dried material by reflux with 70% ethanol (1:10), for two hours. The hydroalcoholic extract was filtered through a textile filter, and used as a stock extract for further analyses.

The fluid hydroalcoholic extract of *Hypericum perforatum* (St John`s Wort) flowering aerial parts (HPEx) was obtained from powdered dried material by reflux with 70% ethanol (1:10), for two hours. The hydroalcoholic extract was filtered through a textile filter, and used as a stock extract for further analyses.

1. Qualitative analyses. The qualitative analysis consisted in phytochemical screening and spectroanalytical profile by HPTLC and UV/VIS absorption spectroscopy.

Phytochemical screening was done by specific chemical reactions for the identification of phytochemicals presence in the analyzed hydroalcoholic extracts (Ciulei *et al*., 1994).

Spectroanalytical profile was done in order to detect the presence of flavonoids represented by rutin and hyperoside (from flavone O- glycosides class) and polyphenolcarboxylic acid (chlorogenic and caffeic acids).

Flavonoids and polyphenols - Equipment: CAMAG LINOMAT IV, WINCATS Planar Chromatography Manager. Chromatographic conditions: Stationary phase - HPTLC plates G60F254 10 x 10 cm, 0.2 mm thickness (Merck); Mobile phase - ethyl-acetate: formic acid: glacial acetic acid: water = 20:2.2:2.2:5.4 v/v; Derivatization- 1% methanol diphenylboryloxyethylamine (Natural Product, NP) followed by 5% polyethylene glycol - 4000 (PEG) in methanol; References - rutin: chlorogenic acid: hyperoside: caffeic acid; Visualization - 254 nm (before derivatization) and 366 nm (before and after derivatization).

Antioxidant activity - Equipment: CAMAG LINOMAT IV, WINCATS Planar Chromatography Manager. Chromatographic conditions: Stationary phase - HPTLC plates G60F254 10 x 10 cm, 0.2 mm thickness (Merck); Mobile phase – ethyl - acetate: formic acid: glacial acetic acid: water = 20:2.2:2.2:5.4 v/v; Derivatization – 0.2% DPPH in methanol; References – rutin: chlorogenic acid: hyperoside: caffeic acid; Visualization – five days after derivatization.

Detection of rutin - Equipment: CAMAG LINOMAT IV, CAMAG TLC 3 Scanner, WINCATS Planar Chromatography Manager. Chromatographic conditions: Stationary phase - HPTLC plates G60F254 10 x 10 cm, 0.2 mm thickness (Merck); Wavelength - 366 nm (after derivatization); Mobile phase – ethyl - acetate: formic acid: glacial acetic acid: ethyl – methyl - ketone: water = 25:3.5:1.5:15:5 v/v; Derivatization - 1% diphenylboryloxyethylamine (Natural Product, NP) in methanol, followed by 5% polyethylene glycol - 4000 (PEG) in methanol; Reference - rutin.

Detection of hyperoside - Equipment: CAMAG LINOMAT IV, CAMAG TLC 3 Scanner, WINCATS Planar Chromatography Manager. Chromatographic conditions: Stationary phase - HPTLC plates G60F254 10 x 10 cm, 0.2 mm thickness (Merck); Wavelength - 366 nm after derivatization; Mobile phase - ethyl acetate: methanol: water: formic acid = 25:1:1.5:3 v/v; Derivatization - 0.5% diphenylboryloxyethylamine (Natural Product, NP) in ethyl acetate, followed by 5% polyethylene glycol - 4000 (PEG) in dichloromethane; Reference - hyperoside.

UV/VIS absorption spectra of extracts were done with a CARY 50 UV/VIS spectrophotometer, by reading each extract - maximum absorption in the 200 - 400 nm range.

2. Quantitative analyses

Flavonoid content of each extract was determined by following colorimetric aluminum chloride method. Briefly, 3 ml solution of each plant extract was diluted to 10 ml with 70% ethanol. 1 ml sample of diluted hawthorn extract and 0.5 ml of diluted St John`s Wort extract were separately mixed with 5 ml of 100 g/l sodium acetate and 3 ml of 25 g/l aluminum chloride, and completed with 50% v/v ethanol at 25 ml. After 15 minutes at room temperature, the absorbance of reaction mixture was measured at 430 nm with a CARY 50 UV/VIS spectrophotometer. Total flavonoid content was calculated as rutin (g/100 ml).

Total polyphenol compounds content were determined by Folin- Ciocalteu method. Briefly, 1 ml solution of each plant extract was diluted to 10 ml with 70% ethanol. 0. 1 ml of each diluted extract were mixed with 7.9 ml distilled water and 0.5 ml Folin - Ciocalteu reagent. After 5 minutes, 1.5 ml of 20% sodium carbonate were added. The absorbance of reaction mixture was measured at 760 nm with a CARY 50 UV/VIS spectrophotometer, after 2 hours of incubation at room temperature. Total polyphenol content was calculated as gallic acid (g/100 ml), which is a common reference polyphenolic compound.

Statistical analysis

Results were evaluated statistically and presented as mean of three determinations ± SD (standard deviation).

RESULTS AND DISCUSSION

1. Qualitative analyses

Phytochemical analysis carried out showed the presence of tannins, flavonoids, polyphenols, and flavonoid glycosides in both extracts. Phytochemical screening also revealed some differences between these two analyzed extracts: reducing sugars, aminoacids and cardiotonic heterosides in St John`s wort; anthocyanosides in hawthorn extract (Table 1).

Table 1. Phytochemical screening of hawthorn (CMEx) and St. John`s wort (HPEx) extracts

Phytochemicals	CMEx	HPEx
Hydroalcoholic extract		
Tannins	+	+ +
Reducing sugars	-	+ + +
Alcaloids	-	-
Aminoacids	-	+
Flavonoids	+ + +	+ +
Polyphenols	+	+
Hydrolised hydroalcoholic extract		
Anthracyanosides	-	-
Coumarins	-	-
Cardiotonic heterosides	-	+
Sterolic saponins	-	-
Triterpens	-	-
Flavonoid glycosides	+	+
Proanthocyanidols	-	-
Anthocyanosides	+ (?)	-

“+” = present; “-“= absent.

The HPTLC images indicate that all references and samples constituents were separated. References were identified at the following Rf values: 0.49 (rutin), 0.56 (chlorogenic acid), 0.71 (hyperoside) and 0.97 (caffeic acid). By comparison of samples with references, constituents of sample extracts were identified. Visualization at 254 nm before derivatization indicated the presence of some dark – grey spots corresponding to flavonoid compounds, in both extracts. Visualization at 366 nm before derivatization indicated the presence of some blue – fluorescence spots corresponding to polyphenolcarboxylic acids, in both extracts. Visualization at 366 nm after derivatization indicated the followings: in case of hawthorn extract, the chromatogram shows two strong, orange fluorescent zones in the region of the hyperoside standard due to hyperoside (Rf = 0.70) and a flavonol monoglycoside (Rf = 0.75), a strong blue fluorescent zone in the region of the chlorogenic acid standard due to chlorogenic acid (Rf = 0.58) and neochlorogenic acid (which are not separated), a weak orange zone of rutin (Rf = 0.48) and a blue fluorescent zone of caffeic acid (Rf = 0.94). Rutin goes together with vitexin- 2″- O- rhamnoside, and hyperoside with vitexin. Rutin and hyperoside are O- glycosides, vitexin and vitexin- 2″- O- rhamnoside are C- glycosides. In case of St John`s Wort extract, the chromatogram shows the flavonoids, rutin (Rf = 0.48) and hyperoside (Rf = 0.72) and other flavonoid zones. The chlorogenic acid (Rf = 0.59) and neochlorogenic acid (Rf = 0.64) give a light blue fluorescence. Discoloration of the characteristic flavonoid compounds and polyphenolcarboxylic acids – spots after derivatization with methanolic DPPH indicated the antioxidant activity of both extracts.

The HPTLC profiles detected the flavonoid compounds represented by rutin (Fig. 3, 4) and hyperoside (Fig. 5, 6) in the hawthorn and St John`s Wort - 70% hydroalcoholic extracts.

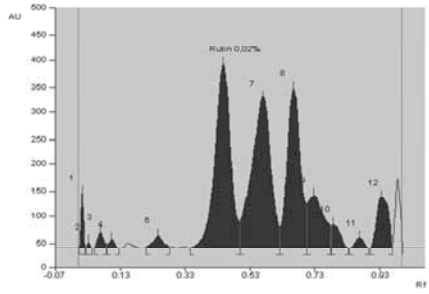


Fig. 3. Rutin detection in hawthorn extract

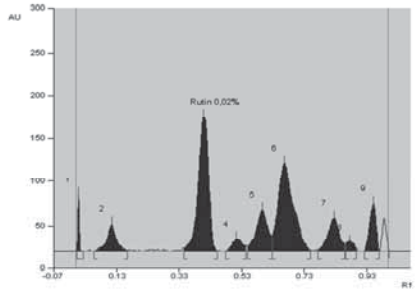


Fig. 4. Rutin detection in St John`s Wort extract

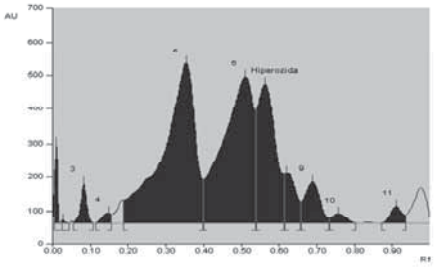


Fig. 5. Hyperoside detection in hawthorn

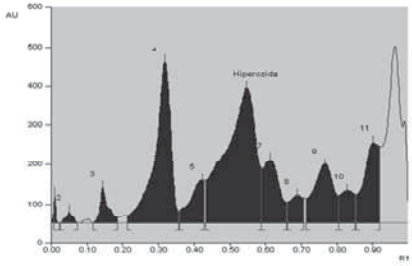


Fig. 6. Hyperoside detection in St John`s

extract Wort extract

The UV/VIS absorption spectra evidenced maximum peaks at 201.1, 205, 207.1, 210, 213, 214.9, 219, 272 and 326.9 nm (Fig. 7) in hawthorn hydroalcoholic extract, and 207.1, 210, 213, 214.9, 218.1, 221.1, 225, 229.1, 235 and 257 nm (Fig. 8) in St John`s Wort hydroalcoholic extract. According to literature, the peaks in the 210 - 310 nm range are due to the phenolic group, and those in the 255 - 280 nm range are specific to the flavonoids. Also, the 255 - 270 nm range are due to the aromatic structures and the chromopherous groups >C=O (Manole, 2008).

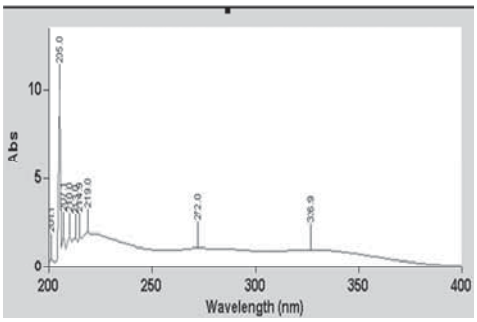


Fig. 7. UV/VIS spectrum of the 70% hydroalcoholic extract of *Crataegus monogyna* leaves and flowers

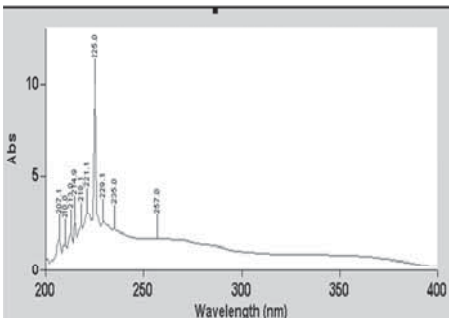


Fig. 8. UV/VIS spectrum of the 70% hydroalcoholic extract of *Hypericum perforatum* aerial parts

Total content of flavonoid compounds (as rutin) and polyphenols (as gallic acid) in red clover stock extract is presented in Table 2.

Table 2. Total polyphenol and flavonoid content of the analyzed hydroalcoholic extracts of *Crataegus monogyna* leaves and flowers and *Hypericum perforatum* aerial parts

Extract	Polyphenols (g gallic acid equivalent/100 ml)	Flavonoids (g rutin equivalent/100 ml)
Hawthorn extract	0.4297 ± 0.0092*	0.1800 ± 0.0016*
St. John`s wort extract	0.5661 ± 0.0125*	0.3538 ± 0.0043*

* = mean of three determination ± SD (standard deviation)

CONCLUSION

Our investigations – qualitative (phytochemical screening, UV/VIS absorption spectra, HPTLC profiles, and antioxidant activity) and quantitative (total flavonoid and polyphenol contents) - concerning the chemical composition of hydroalcoholic extracts of *Crataegus monogyna* Jacq. leaves and flowers, and *Hypericum perforatum* L. aerial parts showed the presence of flavonoid compounds and polyphenolcarboxylic acids, and an antioxidant activity of both extracts. The hydroalcoholic extract of *H. perforatum* had a higher amount of flavonoids and polyphenols.

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