PHYTOCHEMICAL STUDIES ON *CHENOPODIUM BONUS HENRICUS* L. (*CHENOPODIACEAE*)

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Received November 19, 2008

Chenopodium bonus henricus L., named sheperd's spinach, is a spontaneous, nitrophil plant, used for long time in medicinal romanian tradition. The scientific basis for using this plant was perfomed by phytochemical studies. The qualitative and quantitative phytochemical studies were performed using chromatographic (TLC – densitometry, HPLC, GC) and spectral (UV-Vis, MS, AAS) methods.The qualitative and quantitative analysis shown the presents in this plant of the following active compounds: polyphenols – caffeic acid derivatives, flavonoids, tannins; triterpenic saponins; carotenoids; ecdysteroids; fatty oil and minerals.

Key words: *Chenopodium bonus henricus* L.; Chromatographic and spectral methods; Polyphenols; Triterpenic saponins; Ecdysteroids.

INTRODUCTION

Chenopodium bonus henricus L. belongs to *Chenopodiaceae* family and it is growing spontaneously in Romania. It is used due by its cicatrizing, anti-arthritis and anti-cough properties. The nutritional value is due by its high protein and iron content[1].

The plant was used for long time in Romanian tradition, but it wasn't studied to determine its chemical composition.

The plants have complicated composition and the phytochemical studies are performed using chromatographic and spectral methods, methods that can separate and characterize the different compounds from complex matrix[2,3].

This paper presents the phytochemical studies made on different parts of *Chenopodium bonus henricus* L.: herb, stems, leaves, flowers, roots and fruits.

MATERIALS AND METHODS

The plant was harvested near Cluj, was dried, each part separately, and than minced.

The polyphenols were identified and quantified from herb, flowers, leaves and stems, from methanol extracts.

The identification and individual quantification of caffeic acid derivatives were performed by TLC-densitometry. The TLC analysis was performed using: silica plates (Merck) with fluorescence indicator to 254 nm; a mixture of toluene – methyl acetate – formic acid (50:40:10, v/v) as mobile phase[4]; caffeic acid (Roth) and chlorogenic acid (Fluka) as standards. The visualization of chromatogram was made directly on 254 nm and in fluorescence at 365 nm after spraying with Neu-PEG reagent. The densitograms was obtained with a Desaga CD60 photodensitometer at 254 nm. The quantification of chlorogenic acid by TLC-densitometry was performed using a calibration curve of chlorogenic acid obtained in same chromatographic conditions.

The quantification of total polyphenols was performed by UV-Vis spectral method[5] at 500 nm, using Arrnow reagent and calibration curve method. The total polyphenols were expressed in caffeic acid.

The flavonoids and the chlorogenic acid were identified using HPLC method[7]. The HPLC method was performed using a Shimadzu HPLC system, silica-C18 column and gradient elution with acetonitril – phosphoric acid (99,9:0,1, v/v) respectively water – phosphoric acid (99,9:0,1, v/v) at 1 ml/minute. The peaks were detected at 330 nm using a diode array detector. These HPLC conditions were used also for quantification of chlorogenic acid.

The quantification of total flavonoids was performed by UV-Vis spectral method[6] at 430 nm, using aluminum chloride reagent and calibration curve method. The total flavonoids were expressed in rutoside.

The tannins were quantified using UV-Vis spectral method[6] at 715 nm, using phosphor-tungsten reagent and calibration curve method. The tannins were expressed in pyrogalol.

The saponins were isolated from herb and roots. The first step of isolation was the extraction with petrol ether and chloroform, than the degreased vegetal material was extracted with methanol. The methanol extract was concentrated and the triterpenic saponins were precipitated from this extract with acetone. The isolated saponins were purified by dissolution and precipitation[8,9].

The isolated saponins were studied as saponosides (aspect, pH, foaming index, and hemolysis). The quantification of saponosides was performed gravimetric. There were identified the saponoside components using TLC: silica plates (Merck) with fluorescence indicator to 254 nm; a mixture of 2-propanol –formic acid – water (80:15:5, v/v) as mobile phase and visualization in visible light after spraying with Liebermann-Bourchard reagent[8,9]. A part of saponosides were hydrolyzed to the aglyca respectively to the sugar part[8,9].

The aglyca were separated in chloroform phase. The aglyca components were identified using TLC: silica plates (Merck) with fluorescence indicator to 254 nm; a mixture of petrol ether – ethyl acetate – benzene – acetic acid (30:18:60:1,5, v/v) as mobile phase and visualization in visible light after spraying with Liebermann-Bourchard reagent[8,9].

The sugar part were separated in water phase and the sugar components were identified by TLC: silica plates (Merck) with fluorescence indicator to 254 nm; upper phase of a mixture from 1-buthanol – acetic acid – water (4:1:5, v/v) as mobile phase; galacturonic acid, glucuronic acid, galactose, fructose, glucose, xilose, rhamnose as standards and visualization in visible light after spraying with thymol – sulfuric acid reagent[8,9].

The ecdysteroids were extracted from herb in ethanol[7]. The qualitative determination was performed using TLC: silica plates (Merck) with fluorescence indicator to 254 nm; a mixture of ethyl acetate – methanol – ammonia (85:10:5, v/v) as mobile phase; 20-hydroxyecdysone as standard and visualization in fluorescence at 365 nm after spraying with vanillin – sulfuric acid reagent[11,12].

The quantitative determination of ecdysteroids was performed by TLC – UV-Vis spectrophotometry. The separated band for ecdysteroids were eluted from plate with ethanol and the solution was quantified at 242 nm using a calibration curve in 20-hydroxyecdysone.

The carotenoids were extracted by repeated extraction from herb using acetone. The extraction was carried out protected from light and were added buthyl-hydroxy-toluene (BHT) as antioxidant respectively sodium hydrogen-carbonate to avoid the epoxy isomerisation. The extracts were saponified to free the carotenoids from ester form and to eliminate the saponificable lipids.

The individual components were identified and quantified using HPLC. The HPLC method was performed using silica-C18 column and a gradient elution with acetonitril – water – ethyl-isopropyl-amine respectively ethyl acetate – ethyl-isopropyl-amine. The carotenoids were detected at 450 nm with a diode array detector[13].

There were evaluated the total carotenoid content by spectrophotometry.

There were evaluated the fatty oil content of fruits. The fatty oil was obtained using two different methods: cold extraction with chloroform respectively Soxhlet extraction with petrol ether. The fatty oil content was evaluated gravimetric after the evaporation of extraction solvent. There were characterized the fatty oils through them chemical index.

There were evaluated also the fatty acids from fruits. The lipids were extracted with methanol, chloroform and potassium chloride. The extracted lipids were trans-methylated. Than the fatty acids were identified and quantified by gas-chromatography – mass-spectrometry. The GC analysis was performed on Crompack GC system using FID detector respectively on GC-MS system using Omegawax 250, 30 m, 0,25 mm, 0,25 μm column, nitrogen as carrier gas and a temperature program from 190 to 260^0 C[14].

The minerals were evaluated from herb after digestion with nitric acid and perchloric acid[15]. The qualitative and quantitative determination was performed by AAS. There were used a Karl Zeiss Jena, Germany atomic absorbtion spectrophotometer. The minerals were determined in flame at 422,7 nm for calcium, 285,2 nm for magnesium, 324,7 nm for copper, 213,9 nm for zinc, 248,3 nm for iron respectively 279,5 nm for manganese[15].

RESULTS AND DISCUSSION

The phytochemical analysis on polyphenols shows that in different parts of plant exists: chlorogenic acid (caffeic acid class), quercetine, robinine, apigenine (flavonoids) and tannins.

The Figure 1 shows the densitogram obtained for herb extract respectively for chlorogenic acid standard. It can be seen that in herb exists chlorogenic acid, R_f at 0.60, identified also by comparison of "in situ" UV-Vis spectra of separated compound and standard chlorogenic acid.

In Figure 2 can be seen the HPLC chromatogram obtained for herb extract. It can be identify on this chromatogram the chlorogenic acid at 5.57 min, quercetine at 12,81 min, robinine at 14.85 min respectively the apigenine at 17.17 min. The identification was also performed by comparison of UV-Vis spectra of standards with those of compounds separated from plant extract.

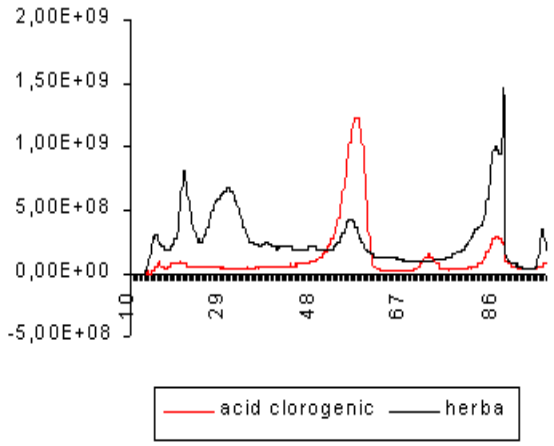

Fig. 1. The densiogram obtained for herb extract respectively for chlorogenic acid standard.

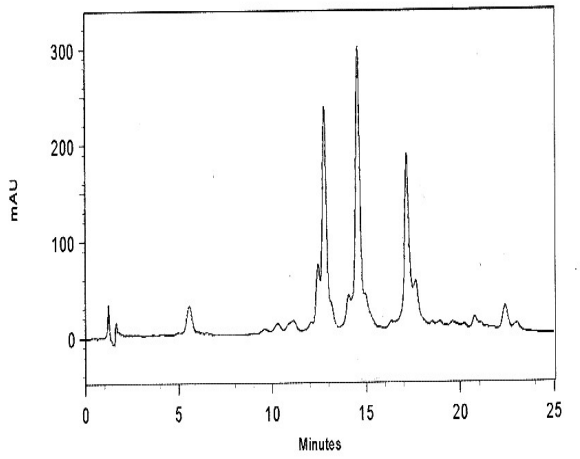

Fig. 2. The HPLC chromatogram obtained for herb extract.

The results of quantification of different polyphenols can be seen in Table 1.

Table 1

The results of quantification of different polyphenols

Polyphenols	Content, % / part of plant			
	Herb	Leaves	Flowers	Stems
Chlorogenic acid, TLC	0.10	0.11	0.16	0.04
Chlorogenic acid, HPLC	0.12	–	–	–
Polyphenols, caffeic acid	0.70	0.73	0.67	0.30
Flavonoids, rutoside	0.92	0.88	0.98	0.30
Tannins, pyrogalol	4.10	–	–	–

The results show that the leaves are rich in polyphenols and the flowers are rich in flavonoids. The chlorogenic acid is one of the most important compounds from caffeic acid class that exist in plant.

The studies upon saponins shown that *Chenopodium bonus henricus* L. contains triterpenic saponins (there were precipitated with acetone). Both saponins from herb and roots are yellowish-white powders, soluble in water and diluted methanol, with pH at 5.5–6.0, foaming index 200 and does not produce hemolysis. In Table 2 are presented the quantification of saponins.

Table 2

The quantification of saponins

Part of plant	Herb	Roots
Content, %	11,6 %	3,18 %

The TLC analysis shows that exists 3 saponoside compounds separated at R_f 0.30; 0.53 respectively 0.83. These compounds are in following proportion: 7.21 %, 84.98 % respectively 7.81 %, that means that exist one main saponosidic compound.

The TLC analysis on aglyca shows that exists 3 different aglyca separated at R_f 0.15; 0.52 respectively 0.57.

The TLC analysis on sugar part identifies, by comparison of R_f values of standards with those of sugars separated from plant, the galacturonic acid, galactose and xilose in herb and the glucose in roots.

The TLC analysis of ecdysteroids had shown the presents of these compounds in herb. The ecdysteroids are separated as a characteristic blue fluorescence band at R_f 0.80, considering the given TLC conditions. The quantification shows the presence in herb of 0.16 % ecdysteroids expressed in 20-hydroxyecdysone.

The Figure 3 shows the HPLC chromatogram of carotenoids from herb. It can be identified, based on UV-Vis spectra and retention time, the following carotenoids: alfa- and beta-carotene, beta-criptoxantine, cis- and trans-luteine and neoxantine, violaxantine and epoxi-beta-criptoxantines.

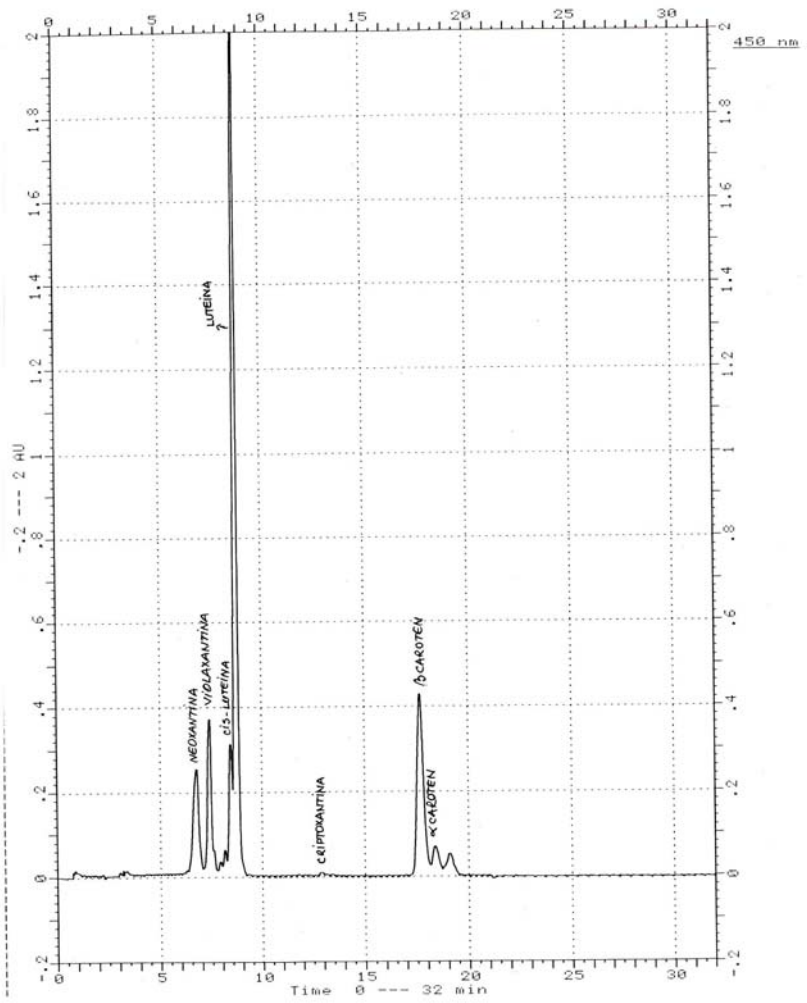

Fig. 3. The HPLC chromatogram of carotenoids from herb.

The quantification of total carotenoids shown a content of 51.3 mg carotenoids in 100 g herb.

The Table 3 shows the composition of carotenoids from herb.

Table 3

The composition of carotenoids from herb

Carotenoids	Content, %
Neoxantine	9.012
Violaxantine	9.213
cis-luteine	7.011
trans-luteine	43.731
beta-criptoxantine	0.281
beta-carotene	17.85
alfa-caroten	3.152
epoxi-beta-criptoxantines	0.125

From these results can be seen that the main carotenoid from *Chenopodium bonus henricus* L. herb is the luteine.

The Table 4 shows the characteristic of fatty oil obtained from fruits. There were obtained 1.84 % fatty oil by cold extraction with chloroform respectively 2.51 % by Soxhlet extraction with petrol ether.

Table 4

The characteristic of fatty oil obtained from fruits

Relative density	0.892	Hydroxyl index	72.400
Refractive index	1.346	Peroxide index	24.770
Acid index	0.890	Saponification index	211.890
Iodine index	140.420	Esterification index	211.000

In Figure 4 can be seen the GC chromatogram of separated fatty acids. There are obtained 14.7 % saturated fatty acids and 84.9 % unsaturated fatty acids. It can be separated: palmitic acid 16:0 (1), palmitoleic acid 16:1 (2), stearic acid 18:0 (3), oleic acid 18:1 (4+5), linoleic acid 18:2 (6), linolenic acid 18:3 (7) and other fatty acids (8,9).

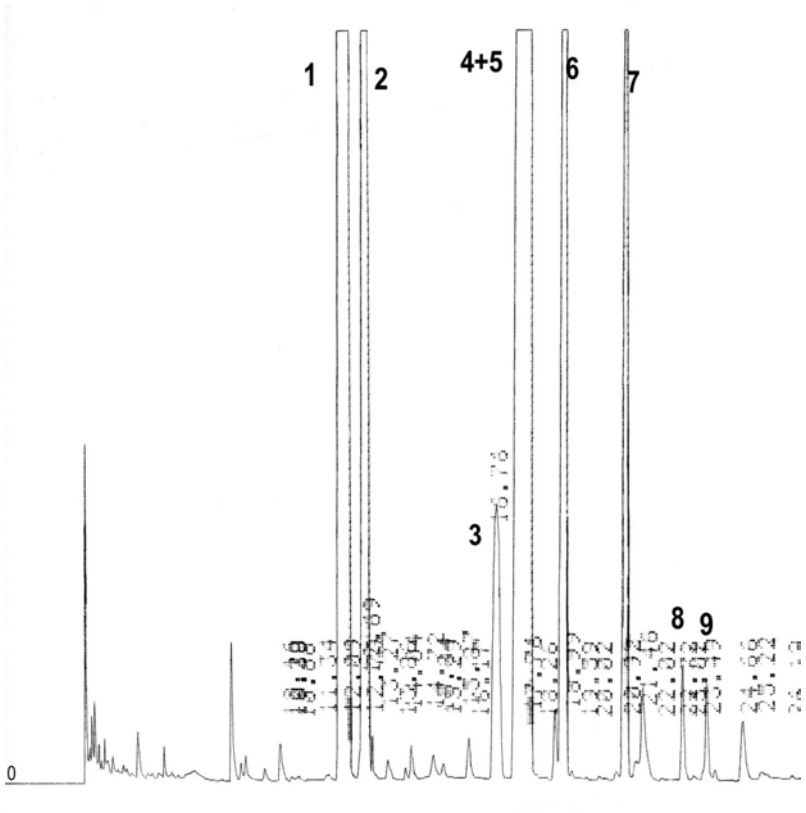

Fig. 4. The GC chromatogram of separated fatty acids.

There can be identified in digested herb the calcium, magnesium, copper, zinc, iron and manganese. The Table 5 shows the content of these minerals in herb.

Table 5

The content of these minerals in herb

Minerals	Content, %
Calcium	0.500
Magnesium	0.168
Copper	0.002
Zinc	0.003
Iron	0.400
Manganese	0.005

The results confirm the high content of plant in iron, but also in calcium and magnesium.

CONCLUSSIONS

The studies show that *Chenopodium bonus henricus* L. contains a lot of therapeutic important compounds like polyphenols, saponins, ecdysteroids, carotenoids (luteine), unsaturated fatty acids and minerals like calcium, magnesium and iron.

Based on these phytochemical determinations the studies can be continued with toxicological and pharmacologic studies that can demonstrate the therapeutic value of this plant.

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