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The phenolic compounds profile of *Sanguisorba officinalis'* roots and herb

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Abstract
The roots and herb of *Sanguisorba officinalis,* traditionally used in medicine as styptic, mild astringent and anti-inflammatory remedy, have been studied for phenolic compounds' qualitative composition and quantitative content by HPLC method. Flavonoids, hydroxycinnamic acids, polyphenols' components and coumarins have been analyzed. Rutin, quercetin-3-D-glucoside, hyperoside, apigenin, umbelliferone, scopoletin, chlorogenic acid, rosmarinic acid, caffeic acid, ferulic acid, gallic acid, gallocatechin, epigallocatechin, epicatechin, catechin gallate, epicatechin gallate, ellagic acid have been determined in *Sanguisorba officinalis* herb. In the herb rutin (0.66 %) predominated among flavonoids, chlorogenic acid (0.59 %) – among hydroxycinnamic acids and epigallocatechin (2.63 %) – among constituents of polyphenols. Apigenin, coumarin, umbelliferone, rosmarinic and *p*-coumaric acids, gallocatechin, epigallocatechin, epicatechin, catechin gallate and epicatechin gallate have been determined in roots of the investigated plant. Content of coumarin in *Sanguisorba officinalis* roots was 0.95 %. Roots of *Sanguisorba officinalis* are characterized by significant content of catechins (gallocatechin, epicatechin, epigallocatechin, catechin gallate, epicatechingallate with the predomination of epigallocatechin (0.9 %)). Roots of the plant contain less flavonoids and hydroxycinnamic acids compared to herb.

Keywords: *Sanguisorba officinalis*, Phenolic ccompounds, Flavonoids, Hydroxycinnamic acids, Components of Polyphenols, High Performance Liquid Chromatography.

1. Introduction
For today standardization of medicinal plant raw material containing biologically active substances of phenolic origin is being performed mostly after total content of phenolic compounds recalculating on separate compounds determined in preliminary component analysis (e.g. TLC). Antioxidant and anti-inflammatory properties of phytomedicines are associated with actions of the phenolic compounds' totality of plant origin. However more specific pharmacological effects, like – antihaemorrhage, astringent, antineoplastic, immune-modulating, sedative, cholagogue, depend on proper separate biologically active substances including those with phenolic structure. Consequently, the study of qualitative composition and quantitative content of individual phenolic compounds is of great importance for the integrated investigation of phytochemistry and pharmacology of plant raw materials and phytomedicines.

Greater burnet (*Sanguisorba officinalis* L.) is well known medicinal plant distributed on territories with temperate climate; it is irreplaceable in folk medicine and recognizable by scientific medicine. Burnet species are also used as ornamental plant due to prolonged flowering (from June to September) with dark red flowers connected in oval heads inflorescences [1].

Greater burnet (*Sanguisorba officinalis* L.*, Rosaceae* family) is perennial herbaceous plant with thick horizontal rhizome and numerous roots.

Sanguisorba officinalis is stated to possess astringent, styptic, antihaemorrhagic and antihaemorrhoidal effects; decoctions and other extracts from *Sanguisorba officinalis* roots are used mainly for metrorrhagia, colitis and diarrhea, haemorrhoids. Also it is listed that burnet's root extract shown antimicrobial, antipyretic, diaphoretic, analgesic, tonic and antistress properties [2, 3].

At the same time, there is a lack of *Sanguisorba officinalis* comprehensive phytochemical researches results in scientific publishing. Phenolic compounds' profile is considered to comment most burnet raw material's pharmacological properties that are documented.

Fig 1: *Sanguisorba officinalis* L.

2. Materials and methods

2.1 The objects: Qualitative composition and quantitative content of phenolic compounds in herb and roots of *Sanguisorba officinalis* were studied. The herb was collected during mass flowering period (in July, 2015), roots were dug out after aerial part had been lied down (in October, 2015) in Western Ukraine (Ternopil region). Plant raw materials were dried at temperature 50±5 ^{0}C in electric dryer.

2.2 The sample preparing: the sample of plant raw material was grinded into a powder by laboratory mill, then about 1.00 g (accurately mass) was selected and placed into the round-bottomed flask (volume 100 ml). The sample was extracted with 50 ml of 60% methanol solution: the flask was attached to the reflux condenser and heated with mixing (on magnetic mixer) at 70 ^{0}C for 30 minutes. Then the sample was treated with ultrasound for 10 minutes at a frequency of 45 kHz at 70 °C. The obtained extract was cooled and quantitatively transferred into the volumetric flask (volume 100 ml), the volume was supplemented to the mark by 60% methanol solution. The constituents of polyphenols were extracted from plant raw materials using 50 ml of bidistilled hot water, heating at temperature 80 °C for 30 minutes. The volume was enhanced up to the mark by bidistilled water.

The obtained sample solutions were colled, carefully mixed, filtered through a membrane filter with a pore size of 0.45 μm and placed into a vial [4, 5].

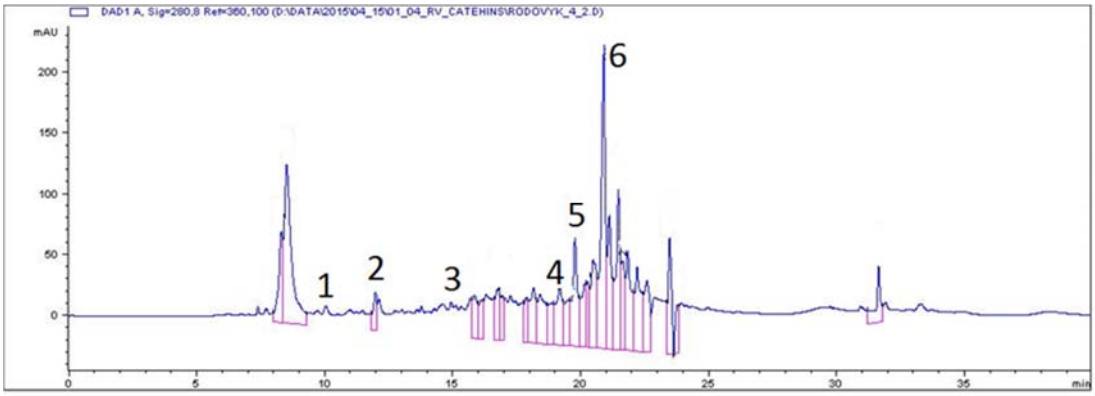
2.3 The essence of the method: chromatographic separation was performed on liquid chromatograph (HPLC method) (Agilent 1200 3 D LC System Technologies, USA) with photometric diode-array detector UV-Vis G1315C equipped with a flow degasser G1322A, autosampler G1329A, column thermostat G1316A, in complex with PC software Agilent Chem Station (G2215 BA). Conditions of chromatography: column Supelco-Discovery C18 size 250 x 4,6 mm with sorbent - silica grains with a diameter of 5 mm, eluants: (A) 0.005 mol/L phosphoric acid (Fluka), (B) acetonitrile; gradient elution mode. The rate of mobile phase: 0.1 ml / min (constituents of polyphenols), 0.8 ml / min (flavonoids), 0.7 ml / min (hydroxycinnamic acids and coumarins), column thermostat temperature of 25 °C. The total duration of analysis 40 minutes (constituents of polyphenols), 50 minutes (hydroxycinnamic acid) 60 minutes (flavonoids and coumarins) [6-9]. Detection range 190-400 nanometers. The wave lengths 255 nanometers and 280 nanometers for constituents of polyphenols, 255 nanometers and 340 nanometers for flavonoids and coumarins, 320 nanometers and 330 nanometers for hydroxycinnamic acids were used.

Phenolic compounds were identified after retention time and UV-spectra values in comparison with standard data; quantification of individual phenolic compounds was determined after the chromatographic peak area depending of their mass concentration [10-13].

3. Results and discussions

Herb and roots of *Sanguisorba officinalis* have been analyzed for phenolic compounds quantitative composition and qualitative content by HPLC method. The study elucidated that *Sanguisorba officinalis* herb contains 5 flavonoids (flavonol kaempferol; flavon apigenin; glycosides of flavonols - rutin, hyperoside, quercetin-3-D-glycoside), 2 simple coumarins (umbelliferone and scopoletin), 4 hydroxycinnamic acids (chlorogenic, rosmarinic, caffeic and ferulic) and 7 constituents of polyphenols (gallic acid, gallocatechin, epigallocatechin, epicatechin, catechin gallate, epicatechin gallate, ellagic acid). The investigated roots contained 1 flavonoid compound (apigenin), 2 simple coumarins (coumarins, umbelliferone), 2 hydroxycinnamic acids (rosmarinic and ferulic) and 5 catechins (gallocatechin, epicatechin, epigallocatechin, catechin gallate, epicatechingallate).

HPLC-chromatogram of the constituents of polyphenolic compounds of *Sanguisorba officinalis* herb is represented in Figure 2. Quantification results of the identified compounds are shown in Table 1.



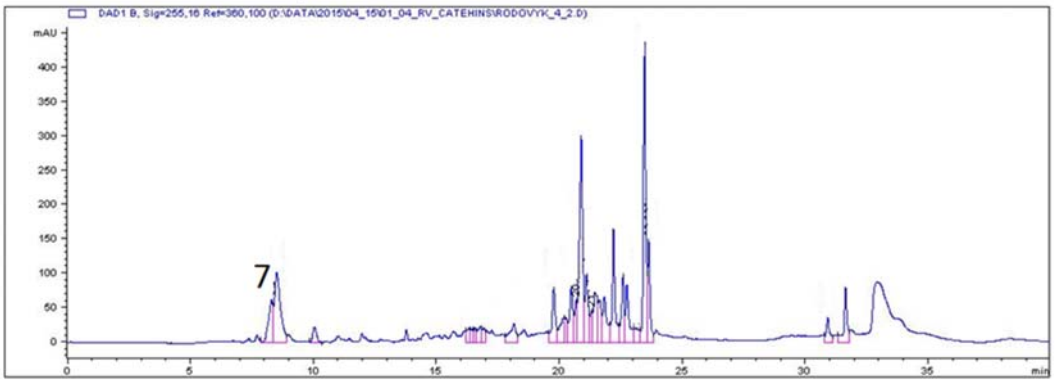


Fig 2: HPLC-chromatogram of the constituents of polyphenols in the *Sanguisorba officinalis* L. herb: a (λ= 280 nm): 1 – gallocatechin, 2 – gallic acid, 3 – catechin gallate, 4 – epigallocatechin, 5 – epicatechin, 6 – epicatechin gallate; b (λ=255 nm): 7 – ellagic acid.

Table 1: Profile of phenolic compounds in *Sanguisorba officinalis* L. raw materials (HPLC)

Phenolic compounds	The content in plant raw materials of *Sanguisorba officinalis* L., %	
	Roots	Herb
Flavonoids		
Hyperoside	-	0.04
Rutin	-	0.66
Quercetin	-	-
Quercetin-3-D-glycoside	-	0.21
Luteolin	-	-
Kaempferol	-	-
Apigenin	0.01	0.01
Coumarins		
Coumarin	0.95	-
Umbelliferone	0.03	0.03
Scopoletin	-	0.03
Hydroxycinnamic acids		
Chlorogenic acid	-	0.59
Rosmarinic acid	0.04	0.05
Caffeic acid	-	0.02
Ferulic acid	-	0.06
p-coumaric acid	0.003	-
Constituents of polyphenols		
Gallic acid	-	0.04
Gallocatechin	0.4	0.48
Epigallocatechin	0.9	2.63
Epicatechin	0.15	0.49
Catechin gallate	0.3	0.21
Epicatechin gallate	0.32	1.67
Ellagic acid	-	0.03

Chromatographic profile of phenolic compounds of *Sanguisorba officinalis* herb and roots has shown the diversity of qualitative composition and quantitative content of flavonoids, coumarins, hydroxycinnamic acids and constituents of polyphenols in the investigated plant raw materials. Thus, herb of *Sanguisorba officinalis* is rich in the constituents of polyphenols: gallic acid, gallocatechin, epigallocatechin, epicatechin, catechin gallate, epicatechin gallate, ellagic acid with the epigallocatechin predomination – 2.63 %. *Sanguisorba officinalis* herb also contains small amounts of simple coumarins umbelliferon (0.03 %) and scopoletin (0.03 %) and flavonoids among which rutin predominates (0.66 %). Catechins in *Sanguisorba officinalis* roots are represented by gallocatechin, epicatechin, epigallocatechin, catechin gallate, epicatechingallate with the predomination of epigallocatechin (0.9 %). Also significant content of coumarin was determined for *Sanguisorba officinalis* roots – 0.95 %. Hydroxycinnamic acids are contained in *Sanguisorba officinalis* roots in very small amounts (0.04 % of rosmarinic acid and 0.003 % of *p*-coumaric acid). In *Sanguisorba officinalis* herb total content of hydroxycinnamic acids was determined – 0.72 %; chlorogenic acid predominated (0.59 %).

As a result of the study herb of *Sanguisorba officinalis* is determined as source of phenolic compounds – constituents of polyphenols. Also significant content of catechins was determined in *Sanguisorba officinalis* roots. Up to date catechins are investigated for various biochemical parameters and pharmacological activities. It was reported that catechins possess antioxidant, cardiovascular system protective, tumor-inhibiting and many other useful properties [14-17]. So, *Sanguisorba officinalis* roots are of great interest to further technological and pharmacological researches with prospective possibility of new safe and effective medicines development.

4. Conclusions

The performed study represents the profile of phenolic compounds in *Sanguisorba officinalis* roots and herb (HPLC method). Herb of *Sanguisorba officinalis* contains high amounts of the constituents of polyphenols with the epigallocatechin predomination (2.63 %), flavonoids (totally 0.92 %) and hyfroxycinnamic acids (totally 0.72 %). Based on the obtained results it is possible to characterize *Sanguisorba officinalis* roots as plant raw material rich in catechins (total content is 2.07 %, individual catechins: gallocatechin, epicatechin, epigallocatechin, catechin gallate, epicatechingallate with the predomination of epigallocatechin (0.9 %)). 0.95 % content of coumarins in *Sanguisorba officinalis* roots also makes it significant for further researches.

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