



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2018; 7(6): 600-602

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www.thepharmajournal.com

Received: 26-04-2018

Accepted: 28-05-2018

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Phenolic compounds from *Pimpinella saxifraga* L.

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Abstract

In this study our goal was to investigate the phenolic content of *Pimpinella saxifraga* L. herb. A high-performance liquid chromatographic method has been used for the estimation of tannins and flavonoids in burnet herb, collected from West region in Ukraine. Four flavonoids (rutin, hyperoside, luteolin and quercetin-3-D-glucoside), gallic and ellagic acids were identified and quantified in the analyzed object. The experimental results showed high amount of rutin (0.44%), luteolin (0.28%) and 0.07% and 0.03% for gallic and ellagic acids respectively. The results obtained from the present study, point out that the *P. saxifraga* L. is a potential source of natural free radical scavengers.

Keywords: *Pimpinella saxifraga* L. (burnet), flavonoids, catechins, HPLC

1. Introduction

The genus *Pimpinella* comprises about 150 species, making it among the largest genera in Apiaceae subfamily Apioideae. *Pimpinella saxifraga* L., a perennial Apiaceae (the carrot or parsley family), occurs at grassy and rocky places in temperate Europe extending to Asia [1]. Burnet is a plant that grows from 15 to 60 cm high. The plant has a long, thick taproot with a strong scent. The basal leaves are pinnate with oval and deeply serrated leaflets of different sizes and the stem leaves are alternate and of a different shape. The flowers are tiny with white, yellowish or pink petals and the seeds are small, dark brown and ovate. The plant blooms from May to September.

The root contains an essential oil (up to 0.4%), saponins (1%), bitter substances, tannins, polysaccharides, resin and the coumarins pimpinellin, bergapten and umbelliferone.

Burnet saxifrage has been used for a long time in folk medicine. The herb was used as a diuretic agent and the root – against heart disease, edema and cough. The dried root is said to have an expectorant, anti-inflammatory, antispasmodic and mildly astringent effect. It is used to dissolve mucus in the airways and to treat cough, sore throat, bronchitis and other infections of the upper respiratory tract. Extracts of the herb can be used as a gargle to reduce inflammation in the mouth and throat.

Burnet may also have diuretic properties and can work well for kidney and urinary tract disorders such as cystitis and stones in the urinary tract.

The herb has been used traditionally to stimulate milk production in lactating women. The oil and resin contained in herb are useful to relief flatulence [2-3].

2. Materials and Methods

2.1 Plant material

Pimpinella saxifraga L. herb was collected in nature reserve “Medobory” (Husyatyn region, Ternopil oblast) in June 2016. The herb was dried using conventional methods and then stored in paper bags in dry place.

All chemicals were of analytical grade (>95% purity) and were purchased from Sigma–Aldrich (USA). High performance liquid chromatography (HPLC) method was applied for separation and quantification of catechins, flavonoids and hydroxycinnamic acids.

2.2 HPLC-analysis of catechins and flavonoids.

The analytical HPLC system employed consisted of a high performance liquid chromatograph Agilent 1200 3 D LC System Technologies, USA coupled with a four-channel vacuum degasser G1354 A, autosampler G1329A, autosampler thermostat G1330 B, column thermostat G1316A, diode array detector (G1315C) in complex with PC software Agilent ChemStation (G2215 BA). The separation was achieved on a Discovery C 18, 250 mm x 4.6 mm x 5 µm (Supelco, № 505129) column with the precolumn of 20 mm at 25 °C temperature [4].

The mobile phase consisted of 0.1 % solution of trifluoroacetic acid, 5 % acetonitrile solution and water (solvent A) and 0.1 % solution of trifluoroacetic acid with acetonitrile (solvent B) – analysis of tannic substances components and distilled water with 0.005 N orthophosphoric acid and acetonitrile (solvent A) and 0.005 N orthophosphoric acid and acetonitrile (solvent B) – analysis of flavonoids (Table 1).

Table 1: The gradient elution parameters for flavonoids and tannic substances components

Flavonoids							
Time, min	0	30	33	38	40	41	49-60
Solvent B, %	12	25	25	30	40	80	12
Tannic substances components (catechins)							
Time, min	0	8	10	15	20	25	28
Solvent B, %	100	12	12	25	25	75	100

The flow rate was 0.1 ml/min and the injection volume was 10 µm. The monitoring wavelengths were 255 and 280 nm for catechins and 255 and 340 nm for flavonoids.

Sample preparation. 2.08 g (accurately mass) of burnet herb material were placed into a 50 mL flat-bottomed flask and extracted with 25 mL of water and the flask was joined to the

reflux condenser and heated for 30 minutes with constant stirring. The obtained extracts were cooled and quantitatively transferred into the volumetric flask and the volume was restored to 100 mL with water. Solutions were filtered through a membrane filter with a pore size of 0.45 µm and placed into a vial.

The identification and quantation of each compound was based on a combination of retention time and peak area [5].

3. Results and Discussion

The HPLC chromatogram of extract of *Pimpinella saxifraga* L. herb showed the presence of rutin, hyperoside, luteolin, quercetin-3-D-glucoside as presented in fig. 1. The analyzed extract of the herb revealed the presence of gallic and ellagic acids as depicted in the HPLC chromatogram in fig. 2.

The HPLC chromatogram of the extract of the plant indicated the content of abovementioned compounds in varying amounts as shown in table 2. A small amount of quercetin-3-D-glucoside (0.09%) and hyperoside (0.19%) among flavonoids (table. 2). A remarkable amount of rutin (0.44%) and luteolin (0.28%). The qualitative content of tannins (gallic and ellagic acids) was set as 0.07% and 0.03% respectively (table. 2).

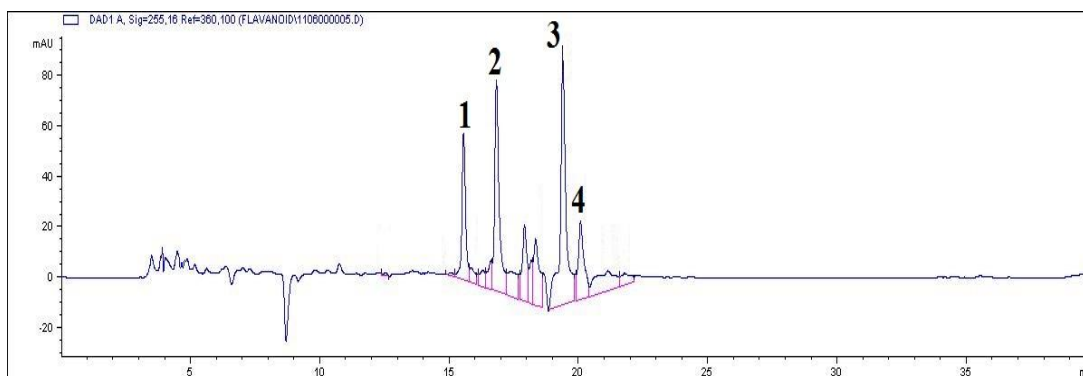


Fig 1: HPLC - chromatogram of flavonoids from *Pimpinella saxifraga* L. herb under wavelength 255 nm: 1- rutin, 2- hyperoside, 3- luteolin, 4- quercetin-3-D-glucoside.

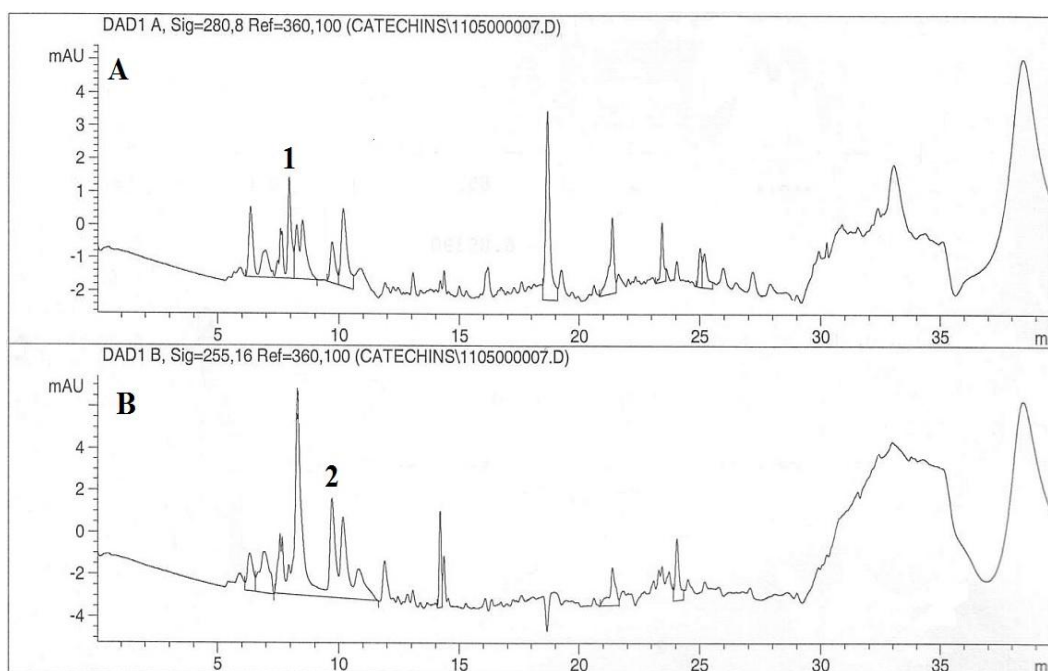


Fig 2: HPLC - chromatogram of catechins from *Pimpinella saxifraga* L. herb: A- under wavelength 280 nm: 1- gallic acid; B- under wavelength 255 nm: 2- ellagic acid.

Table 2: Content of tannins and flavonoids in *Pimpinella saxifraga* L. herb

Compound	Content in dry raw material, %
Flavonoids	
Rutin	0.44
Hyperoside	0.19
Luteolin	0.28
Quercetin-3-D-glucoside	0.09
Tannins	
Gallic acid	0.07
Ellagic acid	0.03

Rutin is a phenolic compound with glycosidic linkage. It is reported to exhibit significant pharmacological activities, including antioxidation, anti-inflammation, antidiabetic etc. It is also used for the treatment of varicose veins, haemorrhoids, haemorrhagic stroke and mucositis. Gallic acid remains either in the Free State or in the combined form as ester and acts as a powerful antioxidant. So the herb of *Pimpinella saxifraga* plant might be considered as an antioxidant agent.

4. Conclusion

In this application note an HPLC method for the analysis and quantitation of two tannins (Gallic and ellagic acids) from *Pimpinella saxifraga* herb was applied. The flavonoid profile of burnet was also established using HPLC method. Flavonoids and tannins were quantified in the analyzed object. The major components were rutin and gallic acid. The presence of significant amount of respective bioactive components in *P. saxifraga* herb ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector.

5. References

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