ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 9, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

RESEARCH OF PHENOLIC COMPOUNDS OF *RUTA GRAVEOLENS* L. AND *STELLARIA MEDIA* (L.) VILL

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Received: 14 March 2018, Revised and Accepted: 27 June 2018

ABSTRACT

Objective: The results of the determination of the phenolic compounds in *Ruta graveolens* L. and *Stellaria media* (L.) Vill. herb by the method of high-performance liquid chromatography (HPLC) with ultraviolet (UV)-detection are presented in the article.

Method: The qualitative composition and quantitative content of the phenolic compounds were studied by HPLC on the Agilent 1200 chromatograph using methanolic extracts of *R. graveolens* L. and *S. media* (L.) Vill. herb. The components of hydroxycinnamic acids were determined at wavelengths of 320 nm and 330 nm; flavonoids at 255 nm and 340 nm; and tannins at 255 nm and 280 nm.

Results: On the basis of the analysis conducted by the method of HPLC in the herb of *R. graveolens* L., 16 substances were identified: 6 flavonoids (apigenin, rutin, quercetin, luteolin, isoquercetin, and hyperoside), 4 hydroxycinnamic acids (chlorogenic, rosmarinic, caffeic, and *p*-coumaric), and 6 tannins (gallic and ellagic acids, gallocatechin, epigallocatechin, epicatechin, and epicatechin gallate). In the herb of *S. media* (L.) Vill., chlorogenic acid, flavone aglycones: Luteolin and apigenin, and flavonol glycosides: Isoquercetin and rutin were identified.

Conclusion: According to the results of the research, it was established that the dominant components of *R. graveolens* L. and *S. media* (L.) Vill. herb are chlorogenic acid and a flavonoid component apigenin, which has anti-inflammatory activity. Thus, the obtained results point to the prospect of further research with the aim of creating of herbal substances with a certain pharmacological action.

Keywords: *Ruta graveolens* L., *Stellaria media* (L.) Vill., Phenolic compounds, Hydroxycinnamic acids, Flavonoids, Tannins, High-performance liquid chromatography.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i9.25920

INTRODUCTION

The search for plants with a sufficient raw material base, which can supplement the nomenclature of officinal species, rational, and complex use of raw materials as well as the creation of new medicines on their basis is an urgent task of modern pharmacy.

The characteristic feature of the representatives of the plant world is their ability to synthesize and accumulate a huge amount of compounds of phenolic origin. For example, the result of study ethanolic extract of *Punica granatum* leaf has showed the maximum amount of total phenols (394.16 mg/g DW of extract), total tannins (210.5 mg/g DW of extract), flavonoids (147.4 mg/g DW of extract), and total triterpenoids (112 mg/g DW of extract) [1]. Natural phenols exhibit high biological activity. They take part in various physiological processes: Breathing, photosynthesis, growth, development, and reproduction of plants; some polyphenols protect plants from pathogenic microorganisms and fungal diseases [2]. The research of the *Millingtonia hortensis* (L). suggests that the leaves contain a considerable amount of phytocompounds such as saponins, alkaloids, flavonoids, and phenols which are responsible for the antioxidant and antibacterial activities. Hence, this plant may be explored as a new source of natural antimicrobial drugs [3].

Drugs based on phenolic compounds are widely used as antimicrobial, anti-inflammatory, hemostatic, choleretic, diuretic, anti-hypertensive, tonic, and astringent agents. Most phenolic compounds of plant origin are low toxic and show no side effects. Prospective are species of plants that have been used long in folk medicine as anti-inflammatory, antiseptic, diuretic, and antispasmodic agents. Such plants are species of *Ruta* L. genus of *Rutaceae* family. Today, about 60 species of the *Ruta* L. genus are described, which are distributed in the Mediterranean and in the temperate zone of Asia. The genus is represented by perennial herbs and shrubs. Two types of the *Ruta* L. genus: *Ruta graveolens* L. and *Ruta divaricata* Ten. grow on the territory of Ukraine. *R. graveolens* L. is cultivated in botanical gardens and farmlands as a decorative, medicinal, and essential oil plant. The herb of *R. graveolens* L. is imported into Ukraine from Iran, Iraq, India, Libya, Algeria, China, and Japan. *R. divaricata* Ten. is common in the foothills of the Southern coast of Crimea [4-6].

Plants of the *Stellaria* L. genus *Caryophyllaceae* Juss Family has been researched. The genus has about 100 species of plants spread all over the globe; in the tropics, representatives of this family occur only in the mountains. In Ukraine, there are 14 species of this genus that occurs all over the territory. The most widespread representative that grows in clogged places, near the houses, like weed in the gardens is *Stellaria media* (L.) Vill. [7,8]. It is believed that this plant improves the activity of the heart and the state of the nervous system, reduces various pain sensations, stops bleeding, heals purulent wounds and affects on tumors of various origin, and exhibits anti-inflammatory, antiseptic, and anti-scorbutic effects. Infusion of herb or juice of fresh plants is used for the treatment of diseases of the liver and gallbladder, lungs (especially in hemoptysis), with bloody vomit, hemorrhoids, as well as with thyrotoxicosis. Strong infusion in the form of lotions is used externally for skin diseases (acne, cuts, rashes, and purulent wounds), in the form of baths, with edema of the legs and nervous excitement. To the medical-prophylactic diet (especially in diseases of the lungs, heart, liver, and kidneys), salads prepared of young fresh leaves and shoots are included [9-11].

The analysis of literature data points to the prospect of a further study of *R. graveolens* L. and *S. media* (L.) Vill. as sources of medicinal plant material.

Considering that one of the sources of biologically active substances obtaining are plants widely used in folk medicine, it is relevant to determine the qualitative composition and quantitative content of phenolic compounds (flavonoids, hydroxycinnamic acids, and tannins) in the herb of *R. graveolens* L. and *S. media* (L.) Vill. to predict the pharmacological action and create new medicines on their basis.

The purpose of the study was to identify and quantify individual compounds in herb of *R. graveolens* L. and *S. media* (L.) Vill. by high-performance liquid chromatography (HPLC).

METHODS

To divide the amount of phenolic compounds by the method of HPLC an Agilent 1200 3 D LC System Technologies (USA) chromatograph equipped with a flow vacuum degasser G1322A, a four-channel pump of a low-pressure gradient G13111A, an auto-sampler (automatic injector) G1329A, a thermostat of columns G1316A, diode-matrix G1315C, and refractometric G1362A detectors were used.

Sample preparation for the determination of *hydroxycinnamic acids* and *flavonoids*: 1.0 g (exact weight) of the grounded raw material was placed in 100 ml round bottom flask, extracted for 15 min with 50 ml of 90% methanol. After this, the sample was treated with ultrasound for 10 min and filtered from the particles of the raw material, and the filtrate was transferred quantitatively into 100 ml volumetric flask, and the volume of the solution was adjusted to 100 ml with 60% methanol [8,9]. The studies were carried out by the reversed-phase chromatography using a Supelco Discovery C18 250 × 4.6 mm chromatography column with a silica gel sorbent modified with octadecyl groups, with grains diameter of 5 μm. The solvent A – 0,005 N orthophosphoric acid and solvent B – acetonitrile were used for determinating hydroxycinnamic acids and flavonoids as a moving phase. Chromatography mode: maximum speed of the moving phase +0.8 ml/min, operating pressure of eluents 156 bar, the temperature 25°C, amount of a sample 5-20 μl, time of chromatography 50 min. The scan time 0.6 s, the detection range 190-400 nm. Gradient Elution Mode for hydroxycinnamic acids: 0 min. 95% of solvent A, 5% of solvent B; 8 min. 92% of solvent A, 8% of solvent B; 15 min. 90% of solvent A, 10% of solvent B; 30 min. 80% of solvent A, 20% of solvent B; 40 min. 60% of solvent A, 40% of solvent B; 41–42 min. 25% of solvent A, 75% of solvent B; 43–50 min. 95% of solvent A, 5% of solvent B, wavelength 320, 330 nm. Gradient Elution Mode for flavonoids: 0 min. 88% of solvent A, 12% of solvent B; 30 min. 75% of solvent A, 25% of solvent B; 33 min. 75% of solvent A, 25% of solvent B; 38 min. 70% of solvent A, 30% solvent B; 40 min. 60% of solvent A, 40% of solvent B; 41 min. 20% of solvent A, 80% of solvent B; 49 min. 88% of solvent A, 12% of solvent B, wavelength – 255 nm.

Isolation of *catechins* was performed by reversed-phase chromatography using a Supelco Discovery C18 250×4.6 mm chromatography column with a silica gel sorbent modified with octadecyl groups, with grains diameter of 5 μm. As a mobile phase was used, solvent A - 0.1% solution of trifluoroacetic acid, 5% solution of acetonitrile and water with pH=2,08; and solvent B - 0.1% solution of trifluoroacetic acid and acetonitrile. Chromatography mode: maximum speed of the moving phase 0.1 ml/min, maximum operating pressure of eluent 40 kPa, the temperature 25°C, amount of a sample 5-20 μl, time of chromatography 40 min. Gradient Elution Mode: 0 min 0% of solvent B, 8 min. 12% of solvent B, 10 min. 12% of solvent B, 15 min. 25% of solvent B, 20 min. 25% of solvent B, 25 min 75% of solvent B, 28 min. 75% of solvent B, 29 min. 0% of solvent B. The scan time 0.6 s, the detection range 190-400 nm, the wavelengths – 280, 255 nm [12-18].

RESULTS AND DISCUSSION

For identification, separation and quantitative determination of phenolic compounds of *R. graveolens* L. herb HPLC method were used. Determination of the quantitative content of hydroxycinnamic acids was carried out at the wavelengths of 320 and 330 nm (Fig. 1, Table 1).

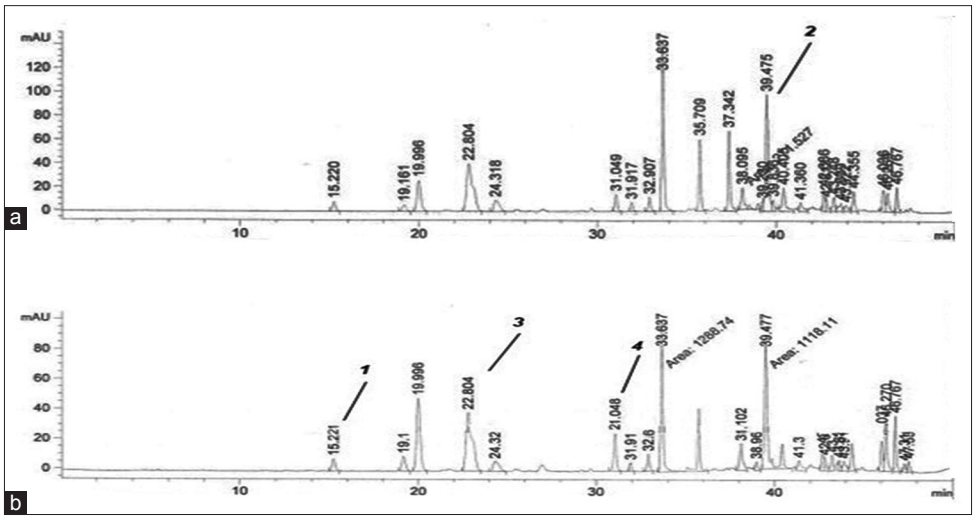

Fig. 1: Chromatogram of hydroxycinnamic acids of *Ruta graveolens* L. herb. (a) The wavelength of 320 nm, (b) the wavelength of 330 nm (1 - carboxylic acid, 2 - rosmarinic acid, 3 - chlorogenic acid, and 4 -– *n*-coumaric acid)

Table 1: The content of hydroxycinnamic acids in *R. graveolens* L. herb

No	Name of the substance	Retention time, s	Concentration	Peak area
1	Chlorogenic acid (5-*O*-Caffeoyl-*D*-quinic acid)	22.804	139.57	990.6
2	Caffeic acid (3,4-Dihydroxycinnamic acid)	15.221	8.47	103.6
3	Rosmarinic acid ([[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl] oxy]-3,4-dihydroxybenzopropanoic acid)	39.475	183.45	1079.1
4	*n*-Coumaric acid (4-Hydroxycinnamic acid)	21.048	14.17	276.7

R. graveolens: Ruta graveolens

According to the obtained data (Table 1), rosmarinic (0.11%), chlorogenic (0.08%), caffeic (0.005%), and *n*-coumaric acids (0.008%) were identified and quantified in the herb of *R. graveolens* L.

Determination of the quantitative content of flavonoids of aglyconic and glycosidic forms was carried out at the wavelengths of 255 and 340 nm (Fig. 2, Table 2).

The results of the conducted studies (Table 2) indicate that the content of rutin is 0.91%, apigenin - 0.01%, quercetin and isoquercetin - 0.01%, luteolin - 0.006%, and hyperoside - 0.02%.

Determination of the quantitative content of tannins (gallic, ellagic acids, and catechins) was carried out at the wavelengths of 280 and 255 nm (Fig. 3, Table 3).

The obtained data (Table 3) show that *R. graveolens* L. herb contains hydrolyzed tannins (gallic [0.01%] and ellagic [0.005%] acids), simple catechins (gallocatechin [0.32%], epigallocatechin [0.52%], epicatechin [0.17%]), and complex catechins (epicatechin gallate [0.06%]).

Determination of the content of hydroxycinnamic acids in *S. media* (L.) Vill. herb was carried out at the wavelength of 320 nm and flavonoids at the wavelength of 340 nm (Fig. 4).

According to the results of the study in the herb of S. media (L.) Vill., chlorogenic acid - 8.12 mg/l, flavone aglycones luteolin - 5.11 mg/l, and apigenin - 12.64 mg/l were identified; such flavonol aglycones as kaempferol and quercetin were not identified, and also such flavonol glycosides as isoquercetin - 2.96 mg/l and rutin 19.99 mg/l were identified.

CONCLUSIONS

By the method of HPLC in the herb of *R. graveolens* L., 16 compounds were identified and quantified, including 4 hydroxycinnamic acids, 6 flavonoids, and 6 tannins. The content of BAS in the herb of *R. graveolens* L. is as follows: Hydroxycinnamic acids: rosmarinic - 0.11%, chlorogenic - 0.08%, caffeic - 0.005%, and *n*-coumaric - 0.008%; flavonoids: rutin - 0.91%, apigenin - 0.01%, quercetin - 0.01%, isoquercetin - 0.01%, luteolin - 0.006%, and hyperoside - 0.02%; and tannins: gallic acid - 0.01%, ellagic acid - 0.005%, gallocatechin - 0.32%, epigallocatechin - 0.52%, epicatechin - 0.17%, and epicatechin gallate - 0.06%. In the herb of S. media (L.) Vill., chlorogenic acid (1.12%), flavone aglycones luteolin (0.11%), apigenin (0.87%), flavonol glycosides isoquercitrin (0.21%), and rutin (0.54%) were identified and quantified.

AUTHOR'S CONTRIBUTIONS

Ms. Melnyk MV - review of literature and data analysis. Mr. Vodoslavskyi VM - review of literature and data analysis. Mr. Obodianskyi MA - data collection.

Table 2: The content of flavonoids in *R. graveolens* L. herb

No	Name of the substance	Retention time, s	Concentration	Peak area
1	Apigenin (5,7,4'-Trihydroxyflavone)	23.90	60.28	537.1
2	Luteolin (5,7,3',4'-Tetrahydroxyflavone)	24.84	5.09	43.32
3	Rutin (3-*O*-β-*D*-rutinoside-5,7,3',4'- Tetrahydroxyflavone)	20.51	732.7	2079.1
4	Quercetin (3,5,7, 3',4'- Pentahydroxyflavone)	25.21	11.9	53.8
5	Isoquercetin (Quercetin-3-*D*-glucopyranoside)	21.67	13.6	68.6
6	Hyperoside (Quercetin -3-*O*-β- galactopyranoside)	25.85	18.1	191.5

R. graveolens: Ruta graveolens

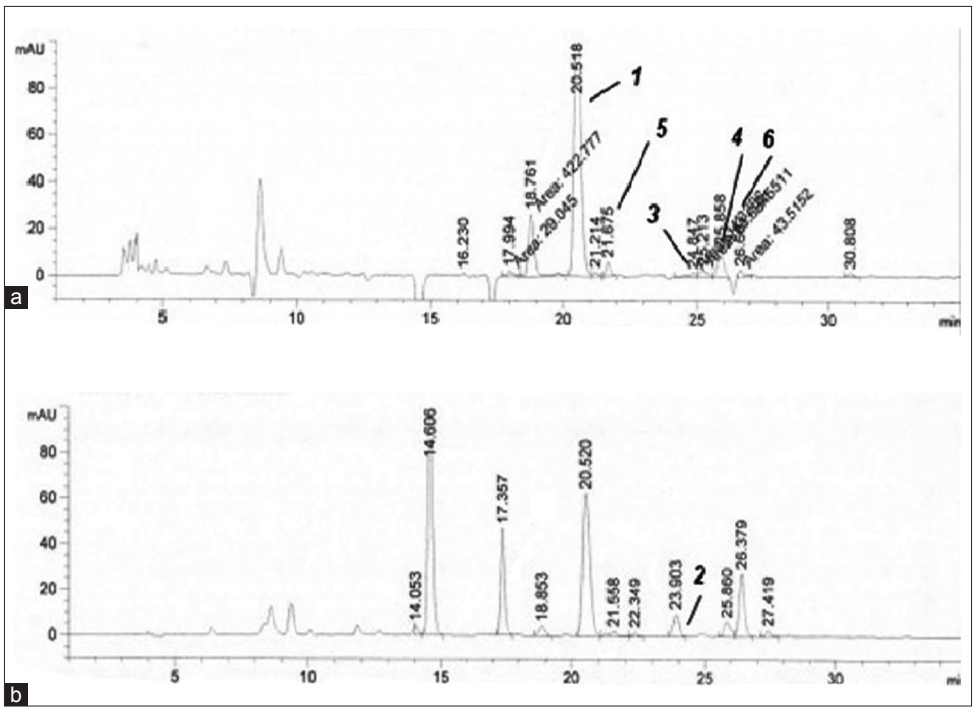

Fig. 2: Chromatogram of flavonoids and coumarins of the *Ruta graveolens* L. herb. (a) The wavelength of 255 nm, (b) the wavelength of 340 nm (1 - rutin, 2 - apigenin, 3 - luteolin, 4 -quercetin, 5 - isoquercetin, and 6 - hyperoside)

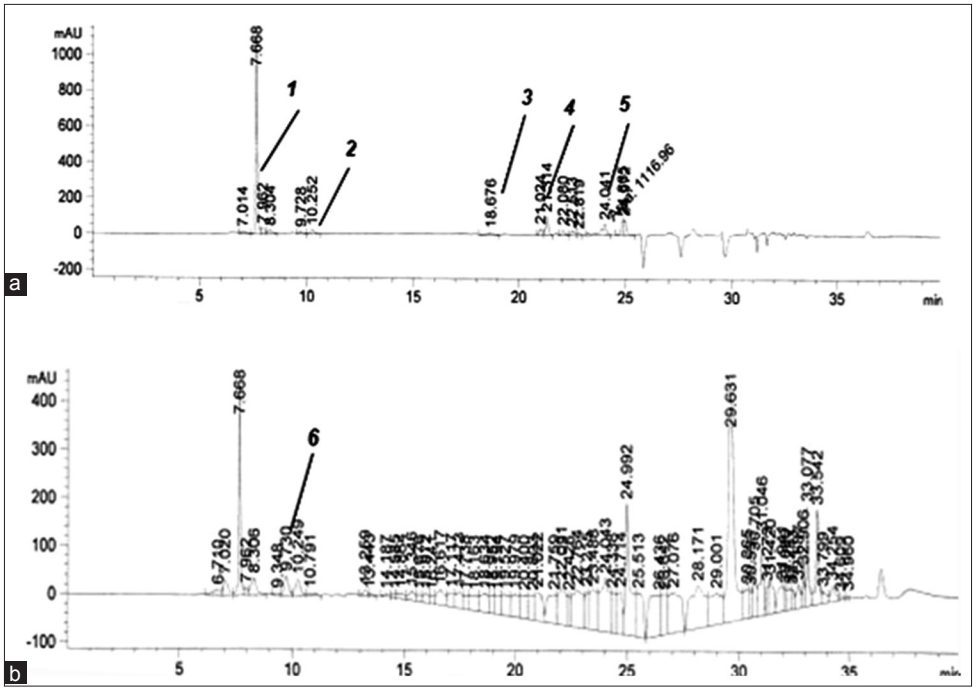

Fig. 3: Chromatogram of tannins of the *Ruta graveolens* L. herb: (a) the wavelength of 280 nm, (b) the wavelength of 255 nm (1 - gallic acid, 2 - gallocatechin, 3 - epigallocatechin, 4 - epicatechin, 5 - epicatechin gallate, and 6 - ellagic acid)

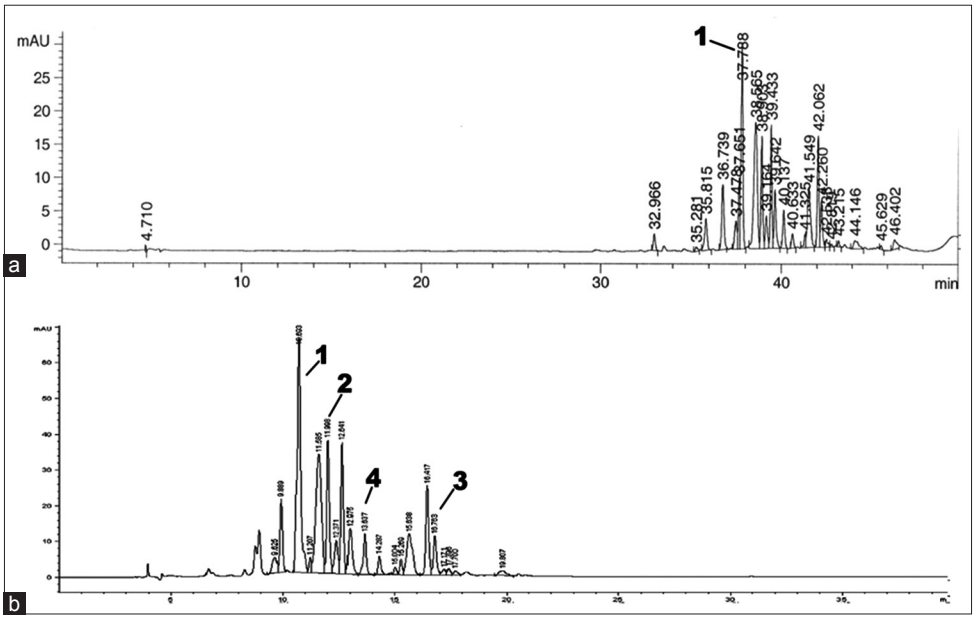

Fig. 4: Chromatogram of *Stellaria media* (L.) Vill. herb. (a) hydroxycinnamic acids, the wavelength of 320 nm (1 - chlorogenic acid); (b) flavonoids, the wavelength of 340 nm (1 - apigenin, 2 - rutin, 3 - isoquercetin, and 4 - luteolin)

Table 3: The content of tannins in *Ruta graveolens* L. herb

No	Name of the substance	Retention time, s	Concentration	Peak area
1	Gallic acid	7.962	18.8	338.7
2	Gallocatechin	10.252	518.7	370.4
3	Epigallocatechin	18.676	835	310.5
4	Epicatechin	21.314	283.3	1012.7
5	Epicatechin gallate	24.041	106.7	1116.9
6	Ellagic acid	9.70	8.10	487.1

R. graveolens: Ruta graveolens

CONFLICTS OF INTEREST

The authors have no conflicts of interest in this study.

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