PHARMACIA

Pharmacia 66(4): 161–164
DOI 10.3897/pharmacia.66.e35194

Research Article

HPLC determination of phenolic compounds content in *Parmelia sulcata* and *Parmelia vagans* thalli

O. A. Kyslychenko[1], Viktoriia V. Protska[1], Iryna O. Zhuravel[1]

1 *National University of Pharmacy, Kharkiv, Ukraine*

Corresponding author: *Viktoriia V. Protska* (vvprotskaya@gmail.com)

Received 5 April 2019 ♦ **Accepted** 30 July 2019 ♦ **Published** 31 December 2019

Citation: Kyslychenko OA, Protska VV, Zhuravel IO (2019) HPLC determination of phenolic compounds content in *Parmelia sulcata* and *Parmelia vagans* thalli. Pharmacia 66(4): 161–164. https://doi.org/10.3897/pharmacia.66.e35194

Abstract

The species of *Parmelia* genus have long been used in Indian folk medicine for the treatment of bronchitis, ulcers, furunculosis, cardiovascular diseases, urolithiasis, amenorrhea, and also at infectious and inflammatory diseases. In Ukraine, the most common lichens of the *Parmelia* genus are *Parmelia sulcata* Tailor and *Parmelia vagans* Nyl. At the same time, thalli of *Parmelia* genus lichens belong to the non-officinal and poorly studied types of raw material.

The qualitative composition and the quantitative content of phenolic compounds in *Parmelia sulcata* and *Parmelia vagans* thalli was studied by HPLC.

According to the results of the chromatographic analysis, salazinic, fumaroprotocetraric, usnic acids, chloratranorin and atranorin were identified in both types of raw material studied. In addition, protocetraric acid was identified in *Parmelia sulcata* thalli.

According to the results of the experiment, the total content of identified phenolic compounds in *Parmelia sulcata* thalli was 2019.71±40.39 g/mol, and in *Parmelia vagans* thalli it comprised 1754.18±34.77 g/mol.

In the thalli of both studied species of *Parmelia* genus, fumaroprotocetraric acid dominanted by the quantity. This substance was present in *Parmelia sulcata* thalli in the amount of 474.00±9.00 g/mol, and in *Parmelia vagans* thalli – 456.21±8.67 g/mol.

In addition, a significant amount of chloratranorin (408.79±8.99 g/mol) was present in *Parmelia sulcata* thalli. Quite a high content of atranorin (393.34±8.65 g/mol) and usnic acid (375.31±7.53 g/mol) were defined in *Parmelia vagans* thalli.

The results obtained can be used in the development of quality control methods for *Parmelia sulcata* and *Parmelia vagans* thalli, as well as medicines based on these types of raw materials.

Keywords

HPLC, lichen, lichen acids, *Parmelia sulcata*, *Parmelia vagans*, phenolic compounds

Introduction

Parmelia Ach. genus includes about 71 species of lichens, which body is built on symbiotic relationship between the hyphae of *Ascomycota* fungi and cyanobacteria (Sharma 2013; Gomez-Serranillos et al. 2014). *Parmelia*

sulcata Tailor. and *Parmelia vagans* Nyl. are among the most widespread species of these lichens in Ukraine (Molina et al. 2011).

The species of *Parmelia* genus have long been used in Indian folk medicine for the treatment of bronchitis, ulcers, furunculosis, cardiovascular diseases, urolithiasis, amenorrhea, and also as an antibacterial, anti-inflammatory and fortifying agent (Cocchietto 2002; Behera et al. 2012; Goyal et al. 2016; Alahmadi 2017; Bondarenko et al. 2017; Kyslychenko et al. 2018; Kyslychenko 2018). The Ayurvedic Pharmacopoeia recommends applying them in cases of skin problems, angina pectoris, ulcers, asthma, dysuria, diarrhea, fever and headache (Goyal et al. 2016).

According to the literature data, these lichens contain polysaccharides, anthraquinones, carotenoids, diterpenoids and steroids, aliphatic acids, amino acids, however, compounds of much interest are the secondary metabolites of phenolic nature – lichen acids, which possess a wide range of biological activity (Sharma 2013; Shcherbakova et al. 2013; Gomez-Serranillos et al. 2014; Bhattacharyya 2016; Studzińska-Sroka et al. 2017).

Lichen acids which are represented by salazinic and protocetraric, fumaroprotocetraric usnic acids, atranorin and chloratranorin are oxygen containing heterocyclic compounds by their structure. They synthetized predominantly in mycobiont mycelium from the primary compounds formed in photobiont cell by photosynthesis. According to the literature data biosynthesis of the compounds mentioned above undergoes acetylpolymalonyl pathway and is initiated by PKS gene. This gene is responsible for the lichen acids precursor (b-orsellinic acid) production. Further, from two to three molecules of this acid through the connection through the ether and carbon-carbon bonds form more complex molecules of lichen acids. Boustie J. and Grube 2005; Elix and Stocker-Wçrgçtter 2018).

They show antimicrobial, antifungal, antiviral, antiprotozoal, antioxidant, antitumor, photoprotective, immune modulating, hypolipidemic, hepatoprotective, anti-inflammatory, analgesic properties (Cocchietto 2002; Behera et al. 2012; Sharma 2013; Shcherbakova et al. 2013; Goyal et al. 2016; Alahmadi 2017; Bondarenko et al. 2017).

The results of the research carried out by Indian scientists have shown lichen acids to possess cardiac protective and hypotensive activity by inhibiting hydroxyl-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and angiotensin-converting enzyme (ACE) (Behera et al. 2012). A group of Saudi Arabian scientists have discovered the solutions of usnic acid in concentration over 100 mmol to have negative inotropic activity on the heart muscle in mice (Gomez-Serranillos et al. 2014).

The literature data indicate the *Parmelia* genus representatives to be prospective in terms of development of new medicinal products with cardiac protective activity. At the same time, *Parmelia sulcata* and *Parmelia vagans* thalli are non-officinal in Ukraine, and their chemical composition has not yet been sufficiently studied.

Aim of the research

The aim of the current research was the determination of qualitative composition and quantitative content of phenolic compounds by HPLC in *Parmelia sulcata* and *Parmelia vagans* thalli.

Materials and methods

The *Parmelia sulcata* and *Parmelia vagans* thalli, which were used for the analysis, were collected at the territory of the Vyshnivchyk forest district of the State Enterprise "Yarmolyntsi forestry" (Khmelnytskyi region, Ukraine) in 2016–2018.

The study of phenolic compounds in the thalli of the analyzed species was carried out using the HPLC method.

The procedure worked out by A. Gudzenko in 2013 was taken as the basis for qualitative composition and quantitative content determination of phenolic compounds in plant raw material.

Sample preparation. 125.0 ml of 70% ethanol were added to 5.0 g of the dried crushed raw material and then heated on a water bath with a reflux condenser for 1 hour. The obtained extract was cooled, filtered through a paper filter with the pore size of 3-5 μm. 5.0 ml of the filtrate were then made up with 70% ethanol to the volume of 10.0 ml (Gudzenko 2013).

The chromatographic analysis of the received samples was carried out using the Agilent Technologies 1200 LC/MSD chromatograph with a diode-matrix mass-elective detector. The stainless steel column C18 SunFire was used in the experiment. Its size was 150 mm × 4,6 mm, and the grain size equaled 3.5 μm. The column temperature was 38°C, the detecting wavelengths of the diode-matrix detector were 254 nm, 330 nm, 350 nm, 360 nm, 370 nm (Gudzenko 2013).

The samples were ionized by electrospray, scanned in the range of 150-800 mass/charge (positive and negative ionization), the injection speed – 1.0 ml/min, the injection volume – 5 μl, eluation – gradient, mobile phase A – 0.1% formic acid solution in water, mobile phase B – 0.1% formic acid solution in acetonitrile (Gudzenko 2013).

Time, min	Mobile phase A, %, (V/V)	Mobile phase B, %, (V/V)
0→1	92	8
1→15	92→70	8→30
15→25	70→0	30→100
25→33	0	100
33→33,5	0→92	100→8

Results and discussion

As a result of the experiment, salazinic, fumaroprotocetraric and usnic acids, atranorin and chloratranorin were identified in *Parmelia sulcata* and *Parmelia vagans* thalli. In addition, protocetraric acid was identified in *Parmelia sulcata* thalli. The HPLC chromatograms of *Parmelia sulcata*

thalli are given in Figure 1, and *Parmelia vagans* thalli – in Figure 2.

The results of the HPLC determination of the quantitative content of phenolic compounds in *Parmelia sulcata* and *Parmelia vagans* thalli are given in Table 1. Both studied types of the raw material accumulated almost equal quantity of phenolic compounds which equaled 2019.71±40.39 g/mol in *Parmelia sulcata* thalli and 1754.18±34.77 g/mol in *Parmelia vagans* thalli.

Fumaroprotocetraric acid dominated by the quantitative content in *Parmelia sulcata* (474.00±09.00 g/mol) and *Parmelia vagans* (456.21±8.67 g/mol) thalli. In addition, chloratranorin accumulated in significant content in *Parmelia sulcata* thalli, which comprised 408.79±8.99 g/mol.

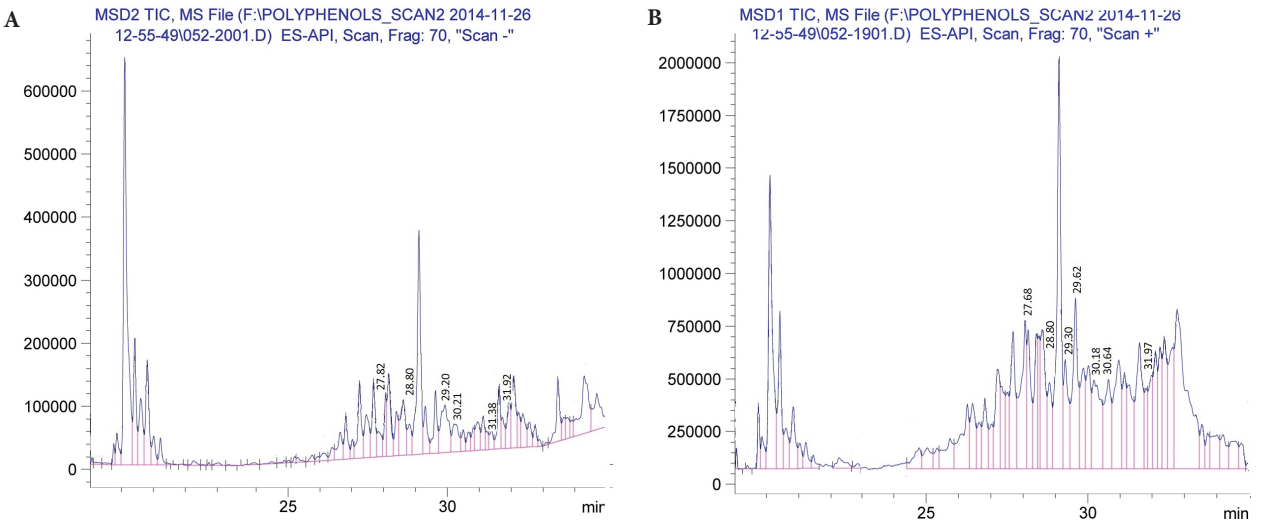

Figure 1. The HPLC chromatograms of *Parmelia sulcata* thalli under the conditions of positive (A) and negative (B) ionization.

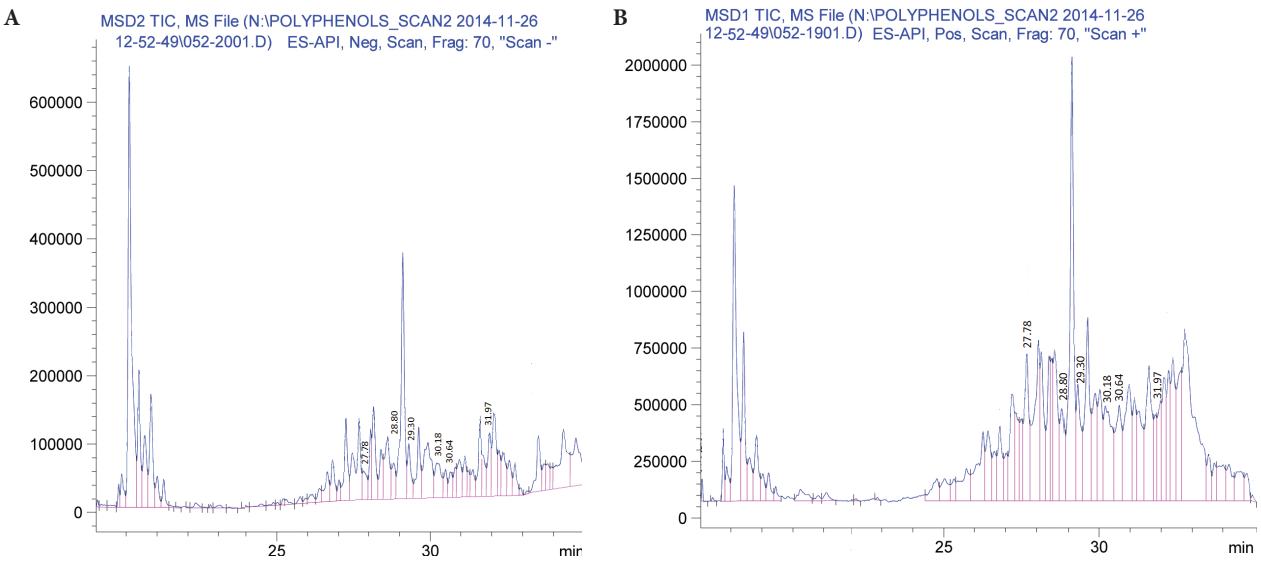

Figure 2. The HPLC chromatograms of *Parmelia vagans* thalli under the conditions of positive (A) and negative (B) ionization.

Table 1. The content of phenolic compounds in *Parmelia sulcata* and *Parmelia vagans* thalli.

Component	*Parmelia sulcata*		*Parmelia vagans*	
	Retention time, min	Quantitative content, g/mol	Retention time, min	Quantitative content, g/mol
Salazinic acid	27.26	388.28±7.77	27.26	349.78±7.01
Protocetraric acid	28.87	374.30±7.11	–	–
Fumaroprotocetraric acid	29.91	474.00±9.00	29.11	456.21±8.67
Usnic acid	30.19	344.32±7.23	29.99	375.31±7.53
Chloratranorin	31.37	408.79±8.99	31.39	270.26±5.68
Atranorin	31.91	374.34±7.49	32.25	393.34±8.65
Total content	2019.71±40.39		1754.18±34.77	

The content of this compound in *Parmelia vagans* thalli was 1.5 times lower than in *Parmelia sulcata* thalli and equaled 270.26±5.68 g/mol. It should be pointed out that the content of chloratranorin in *Parmelia sulcata* thalli was 1.7 times lower than the content of fumaroprotocetraric acid. The content of usnic acid and atranorin was somewhat higher in this type of the raw material – 375.31±7.53 g/mol and 393.34±8.65 g/mol respectively.

The content of usnic acid (344.32±7.23 g/mol) in *Parmelia sulcata* thalli was almost at the same level as the one in *Parmelia vagans* thalli (375.31±7.53 g/mol), but at the same time it was 1.4 times lower compared to the content of fumaroprotocetraric acid, which accumulated in the analyzed raw material in the maximum quantity.

The content of salazinic and protocetraric acids, as well as atranorin in *Parmelia sulcata* thalli was almost equal and comprised 388.28±7.77 g/mol, 374.30±7.11 g/mol and 374.34±7.49 g/mol respectively. The content of salazinic acid in *Parmelia vagans* thalli was 349.78 ±7.01 g/mol, which was 1.3 times lower compared to the content of fumaroprotocetraric acid (456.21±8.67 g/mol) in this type of the raw material.

The maximum content of chloratranorin, salazinic and fumaroprotocetraric acids was observed in *Parmelia sulcata* thalli, while the content of usnic acid and artanorin – in *Parmelia vagans* thalli.

Conclusions

Salazinic, fumaroprotocetraric, usnic acids, chloratranorin and atranorin were identified in both types of the raw materials studied. In addition, protocetraric acid was identified in *Parmelia sulcata* thalli. In the thalli of both studied species of *Parmelia* genus, fumaroprotocetraric acid dominanted by quantity. The content of this substance in *Parmelia sulcata* thalli was 474.00±9.00 g/mol, and in *Parmelia vagans* thalli – 456.21±8.67 g/mol.

As the result of the research it was determined that *Parmelia sulcata* and *Parmelia vagans* thalli had identical qualitative composition of phenolic compounds. The studied objects insignificantly differ by the quantitative content of these compounds.

The obtained results will be used in the development of quality control methods for *Parmelia sulcata* and *Parmelia vagans* thalli, as well as medicines based on these types of the raw materials.

References

Abdulaziz AA (2017) Usnic acid biological activity: history, evaluation and usage. International Journal of Basic & Clinical Pharmacology 6(12): 2752–2759. https://doi.org/10.18203/2319-2003.ijbcp20175072

Behera B C, Mahadik N, Morey M (2012) Antioxidative and cardiovascular-protective activities of metabolite usnic acid and psoromic acid produced by lichen species Usnea complanataunder submerged fermentation. Pharmaceutical Biology 50(8): 968–979. https://doi.org/10.3109/13880209.2012.654396

Bhattacharyya S, Deep PR, Singh S, Nayak B (2016) Lichen Secondary Metabolites and Its Biological Activity. American Journal of PharmTech Research 6(6): 28–44. https://www.researchgate.net/publication/311640971_Lichen_Secondary_Metabolites_and_Its_Biological_Activity

Bondarenko V, Korczynski M, Techathaveewat W (2017) The Antimicrobial Properties of Extracts Isolated from Lichen Parmelia vagans. FASEB journal 31(1): 1–10. http://www.fasebj.org/content/31/1_Supplement/939.13.short

Boustie J, Grube M (2005) Lichens–a promising source of bioactive secondary metabolites. Plant Genetic Resources 3(2): 273– 287. https://doi.org/10.1079/PGR200572

Cocchietto M, Skert N, Nimis GPL, Gianni S (2002) A review on usnic acid, an interesting natural compound. Naturwissenschaften 89: 137–146. https://doi.org/10.1007/s00114-002-0305-3

Elix JA, Stocker-Wçrgçtter E (2018) Biochemistry and secondary metabolites. Lichen Biology. In: Thomas H, Nash III (Eds) Lichen Biology. Cambridge University Press, Cambridge, 104–133. https://doi.org/10.1017/CBO9780511790478.008

Gomez-Serranillos MP, Fernandez-Moriano C, Gonzalez-Burgos E, Divakar PK, Crespo A (2014) Parmeliaceae family: phytochemistry, pharmacological potential and phylogenetic features. RSC Advances 4: 59017–59047. https://doi.org/10.1039/C4RA09104C

Kumar GP, Kumar VS, Kumar SA (2016) Pharmacological and phytochemical aspects of lichen Parmelia perlata: a review. International Journal of Research in Ayurveda and Pharmacy 7(1): 102–107. https://doi.org/10.7897/2277-4343.07138

Gudzenko A (2013) Development and validation of a RP-HPLC method for the simultaneous determination of luteolin and apigenin in herb of *Achillea millefolium* L. The pharma innovation – journal 2(7): 7–14. https://pdfs.semanticscholar.org/2b37/48b3208bb9f5edc270cc13da34bda1f148b9.pdf?_ga=2.180542503.1477516140.1558443214-1313361779.1499770704

Kyslychenko OA, Protska VV, Zhuravel IO (2018a) The study of the mineral composition of parmelia perlata thalli. Medical and Clinical Chemistry 20(1): 117–122. https://doi.org/10.11603/mcch.2410-681X.2018.v0.i1.8754

Kyslychenko OA, Protska VV, Zhuravel IO, Hutsol VV (2018b) The study of volatile compounds of parmelia perlata thallus. Pharmacia 65(3): 11–16. http://bsphs.org/wp-content/uploads/2018/12/Protska.pdf

Molina MC, Divakar PK, Millanes AM, Sánchez E, Del-Prado R, Hawksworth DL, Crespo A (2011) Parmelia sulcata (Ascomycota: Parmeliaceae), a sympatric monophyletic species complex. The Lichenologist 43(6): 585–601. https://doi.org/10.1017/S0024282911000521

Sharma AK, Sharma MC, Dobhal MP (2013) Phytochemical constituents from different species of parmelia genus: A review. Der Chemica Sinica 4(1): 1–11. http://www.imedpub.com/articles/phytochemical-constituents-from-different-species-of-parmelia-genusa-review.pdf

Shcherbakova AI, Koptina AV, Kanarskiy AV (2013) Biologically Active Substances of Lichens. Forest Journal 3: 7–16. http://lesnoizhurnal.ru/upload/iblock/c21/lh1.pdf

Studzińska-Sroka E, Galanty A, Bylka W (2017) Atranorin – an interesting lichen secondary metabolite. Mini-Reviews in Medicinal Chemistry 17: 1633–1645. https://doi.org/10.2174/1389557517666170425105727