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Submitted: 26/09/2025 - Accepted: 23/11/2025 - Published: 28/12/2025

Preliminary Data Regarding Total Chlorophylls, Carotenoids and Flavonoids Content in Flavoparmelia Caperata (L.) Hale Lichens Species

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DOI: 10.26417/2ysor0zk

Abstract

Flavoparmelia caperata L. Hale (common greenshield lichen) is an Ascomycete, foliose lichen usually growing on tree bark in most mesophytic-forested habitats in Romania. Lichen samples were collected from three stations in Romania (Craiova, Timișoara, Hoia-Baciu Forest, Cluj) and extracted in 96% ethanol. Both dry lichen tissue powder and extracts were analyzed by UV-Vis spectrophotometry for determining photosynthetic pigment concentrations (chlorophylls a and b and total carotenoids). Dry tissue samples had chlorophyll a concentrations of 95.12-109.59 mg/kg DW, chlorophyll b concentrations of 88.53-110.98 mg/kg and total carotenoids 88.89-102.85 mg/kg. Alcoholic extracts of fresh lichen tissue showed an extreme variability, with 715.97-10331.50 mg/kg chlorophyll *a*, 527.77-8124.20 mg/kg chlorophyll *b*, 1125.72-8714.90 mg/kg carotenoids pigments, with the highest values in samples from Craiova. A lower variability was observed in flavonoid contents, with 461.78-966.02 mg/kg DW in dry powder and 815.56-2734.135 mg/kg in ethanolic extracts. Extracts of lichens from Craiova had a decreased content of total flavonoids compared with the other two lichens ethanol extracts.

Keywords: *Flavoparmelia caperata* (L.) Hale, total chlorophylls, carotenoids, flavonoids

Introduction

Flavoparmelia caperata (L.) Hale – the common greenshield lichen – is a medium to large-sized, foliose Ascomycete lichen (Lecanorales Order, Parmeliaceae Family) (Fig. 1). With medium to large dimensions (up to 20 cm in diameter), the foliage is green,

turning to a specific yellowish shade when dry. It has a variate color, a lobed shape, with granular soredia. On the back it is blackish, with brown edges. It has rounded, flattened lobes, 6-10 mm, with granular sores, brown apothecia, sessile and unbranched rhizoids, abundant especially on the edge. Like most Parmeliaceae, the phototrophic symbiont is a green algae of the genus *Trebouxia*. It is a mesophytic species, abundant especially in areas with a Mediterranean climate. The greenshield lichen grows on tree bark (rarely on rocks), on various woody species, throughout Mediterranean, sub-Mediterranean and temperate forests (alpine regions excluded) and only exceptionally on the ground. It also prefers an acidic or subacidic *p*H, tolerates direct exposure to sunlight and moderately eutrophic environments [1].

Lichens were used since ancient times, for various folk medicine applications. *Flavoparmelia caperata* is used in some regions of Chile for treating dispepsia, bronchitis, diabtetes, tuberculosis, hemorrhages, spermatorrhea etc.

In Romania it is frequently found in oak forests (on various species of the genus *Quercus*). It can also be found on the bark of maples, birches, lindens, walnuts, carobs, beeches, hornbeams, trees of *Prunus* genus, including the orchards. Sometimes it also colonizes the bark of conifers, the wood of dead trees or the saxicol environment [2].

Regarding the chemical composition of lichen tissues, there are differences related to the age and region of the thallus. Iron and copper are mainly concentrated in symbiotic algae. The lower cortex of the young parts concentrates elements such as Ni, Si, Ti, probably having a protective role. The marrow, especially that of my old areas of the thallus, accumulates calcium oxalate. Non-metals (P, S, Cl, are more abundant in the upper layers) [3].

Flavoparmelia caperata easily accumulates heavy metals in the atmosphere particulate. Due to its abundance and large size, it is a valuable bioindicator of metals pollution. Thus, in areas with industrial activities such as coal-fired power plants or metallurgical plants, there were significant increases in tissue concentration of As (4-39 mg / kg), Ce (7-708 mg / kg), Cr (3-863 mg / kg), Co (0.4-6.8 mg / kg), Fe (790-17.830 mg / kg), La (1.6-236 mg / kg), Ti (87-1850 mg / kg) , V (2-27 mg / kg), Zn (19-141 mg / kg) [4].

Other bioaccumulated elements by this lichens species are B (6-19 mg / kg), Ga and In (less than 0.1 mg / kg), Li (0.4-0.7 mg / kg), Pb (7-22 mg -kg), Sr (11-40 mg / kg), Tl (0.6-1.5 mg / kg) and Zn (17-24 mg / kg). Ba, K, Mg, Na, Tl and Zn elements accumulate especially in the young areas of the thallus, and As, B, Cd, Cr, Cu, Fe, Ga, In, Li, Ni, Pb and Se preferentially in the central areas [5]. After other reported determinations, concentrations of about 25 mg / kg Cu, 5,000 mg / kg Fe, 80 mg / kg Zn, 80 mg / kg Pb can be achieved [6].

On the other hand, it has been found that this species has a medium to low resistance to air pollution, growing abundantly only in unpolluted ecosystems [7].

Recently research emphasized that *Flavoparmelia caperata* contains significant and easily extractable amounts of carbohydrates, proteins, steroids, tannins and triterpenes, as primary metabolites, and atranorine, usnic acid and protocetraric acid, as secondary metabolites [8].

Flavoparmelia caperata contains about 90 mg / g phenolic compounds, 34 mg / g flavonoids and a remarkable antioxidant capacity among other lichen species (550 μg / mL IC50) [9]. Studies have shown that there is a seasonal variation in the content of antioxidants (polyphenols, flavonoids), with minimal values in the vernal season [10].

Usnic acid (about 0.35% by weight) present in this lichen species, has antimicrobial properties, especially against the genus *Mycobacterium* [11]. Also, this compound and other secondary metabolites, have some efficacy in inhibiting α-amylase, with a possible role in diabetes improving [12]. Antimicrobial abilities were also noticed against *Bacillus subtilis, B. cereus*, *Staphylococcus aureus, Enterococcus faecium, Erwinia amylovora* etc. bacteria strains, also against fungi of Aspergillus genus. Antiproliferative properties have been demonstrated, for example against colon adenocarcinoma cells [9, 13, 14].

Other potentially valuable compounds reported in *Flavoparmelia caperata* species are as follows, heavy polysaccharides - homoglucans (lichen, isolichenan, pustulan, evernan, nigeran) and heteroglycans (galactomannan, glucomannan), with antitumor, immunomodulatory, antiviral properties, etc. [15, 16].

Among other valuable active principles in *Flavoparmelia caperata*, chlorophylls (the main photosynthetic pigments) are known for their anti-inflammatory, anti-carcinogenic properties [17 - 19]. They are a source of magnesium and inhibit kidney stone formation. Carotenoids are the precursors of melanin and retinol, key compounds for skin and eye health, also, with antioxidant and antiproliferative properties [19, 20]. Flavonoids (low-mass polyphenolic compounds) are strongly antioxidant, antiviral, antibacterial, antifungal, antiproliferative and antitumoral compounds [26, 27]. The paper reveals the comparative preliminary results regarding the total chlorophylls, carotenoids and flavonoids content determination in *Flavoparmelia caperata* (L.) Hale, collected from three different Romanian pedo-climatic area, in the aim to emphasize the therapeutic potential of this lichens species.

Material and Methods



Fig. 1. *Flavoparmelia caperata* (L.) Hale lichens species [29 - 31]



Fig. 2. Appearance of the *Flavoparmelia caperata* (L.) Hale lichens powder

Sampling:

Lichen samples were collected in the period summer-autumn of 2018-2019, from three sampling sites of Romania, as follows:

- City center Park of Timişoara
- Hoia-Baciu Forest of Cluj-Napoca

„Alexandru Buia" Botanical Garden of Craiova

Part of the lichen samples were air-dried for determining dry biomass percentage and ground to powder using an electrical grinder (Fig. 2). Part of the samples were extracted with 96% ethanol at 1% final concentration for all samples.

Analysis:

For chlorophyll *a*, chlorophyll *b* and total carotenoid determinations, an amount of 1 g ground tissue powder was extracted in 100 mL 80% acetone (triplicate samples for each species). The extract was filtered at normal pressure through Whatman blue band filter paper and the spectrophotometric absorbance was read (using a S106 WPA UV-Vis spectrophotometer) against an 80% acetone blank, at 470 nm, 647 nm and 663 nm of wavelengths [18, 19]. Absorbance values were used to calculate carotenoids pigments concentration, according to the specific trichromatic equations [21 - 25].

For flavonoid determinations, an amount of 1 g ground tissue powder was extracted in 5 mL methanol p.a. and filtered (triplicate samples). An aliquot of 0.5 mL of extract was diluted in 4 mL water and 8 mL methanol mixture, and the spectrophotometric absorbance was read against a methanol blank, at 340 nm wavelength [27, 28].

Results and Discussion

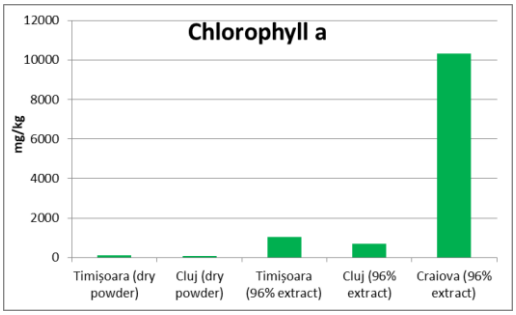


Fig. 3. Average concentration of chlorophyll a in dry powder and ethanolic extracts of Flavoparmelia caperata samples from selected stations

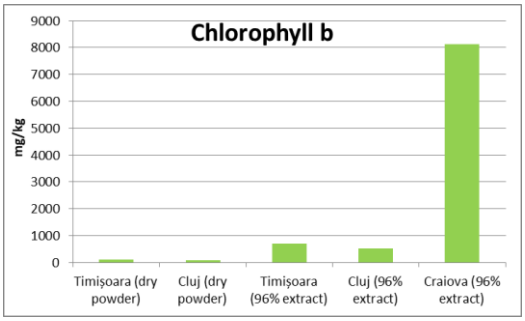


Fig. 4. Average concentration of chlorophyll b in dry powder and ethanolic extracts of Flavoparmelia caperata samples from selected

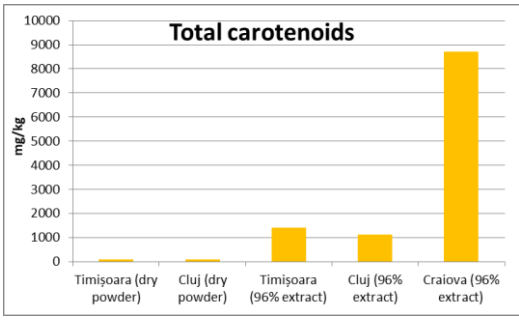


Fig. 5. Average concentration of total carotenoids in dry powder and ethanolic extracts of Flavoparmelia caperata samples from selected stations

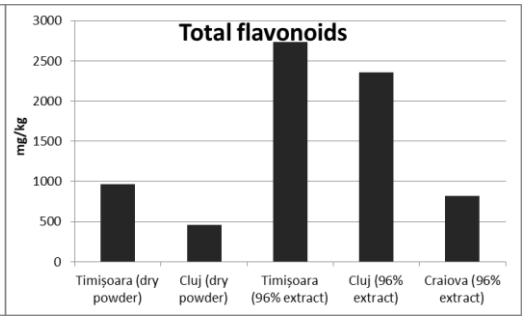


Fig. 6. Average concentration of total flavonoids in dry powder and ethanolic extracts of Flavoparmelia caperata samples from selected stations

Dry tissue samples had chlorophyll *a* concentrations of 95.12-109.59 mg/kg DW, chlorophyll *b* concentrations of 88.53-110.98 mg/kg and total carotenoids 88.89-102.85 mg/kg.

Alcoholic extracts of fresh lichen tissue showed an extreme variability, with 715.97-10331.50 mg/kg chlorophyll *a*, 527.77-8124.20 mg/kg chlorophyll *b*, 1125.72-8714.90 mg/kg carotenoids, with the highest values in samples from Craiova.

A lower variability was observed in flavonoid contents, with 461.78-966.02 mg/kg DW in dry powder and 815.56-2734.135 mg/kg in ethanolic extracts. Extracts from Craiova samples had lower content than other ethanolic extracts.

Conclusion

The preliminary results regarding the total chlorophylls, carotenoids and flavonoids contents of *Flavoparmelia caperata* (L.) Hale lichens species, emphasize a major geographical and pedo-climatic differences with impact on chemical composition.

The significant lower values obtained in the case of lichens drying tissue compared with hydroalcoholic extracts for all four bioactive compounds classes, were highlighted.

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