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QUANTITATIVE ANALYSIS OF SOME CLASSES OF BIOACTIVE ANTIOXIDANT COMPOUNDS IN FENNEL (*FOENICULUM VULGARE* MILL.) LEAVES

Popoviciu Dan Răzvan, Pricop Simona-Mariana*
„Ovidius" University of Constanţa, Faculty of Natural Sciences and Agricultural Sciences, Constanţa, Romania
** Correspondence author. E-mail:* simona.pricop@yahoo.com

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ABSTRACT

Fennel leaves from local production were studied for their content in some classes of compounds with antioxidant potential. Chlorophylls (a and b), carotenoids (total, β-carotene, lycopene, lutein, zeaxanthin and cryptoxanthin), phenolic compounds (total, flavonoids, anthocyanins), ascorbic acid, essential oils.
The chlorophyll content was 1,459 mg/kg DW, of which over 86% chlorophyll a. Total carotenoid concentration was 1,609 mg/kg DW, including 1,002 mg/kg β-carotene, 109 mg/kg lycopene, 32 mg/kg lutein and low amounts of other compounds. 13,292 mg/kg was the total phenolic content, of which 686 mg/kg flavonoids, including 146 mg/kg anthocyanins. Leaves contained 2,658 mg/kg ascorbic acid and 20,553 mg/kg essential oil.
While comparable with other Apiaceae in most types of compounds, fennel had a lower flavonoid inventory, but a higher amount of lycopene.

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill., Apiaceae family) is a herbaceous aromatic plant, native of the Mediterranean region. It has a tall (up to 2.5 m) stem, with a bulb-like base, long (40 cm), pinnately dissected leaves, yellow flowers grouped in compound umbels. Fruits are indehiscent schizocarps.

It is widely cultivated as an aromatic and medicinal plant and, in the last years it is gaining popularity in Romania also. Leaves are strongly aromatic. Stem base (in Florence fennel) is used in cuisine as a vegetable. Fruits are also aromatic and rich in essential oil, used in cosmetic and pharmaceutic products.

Among its medicinal uses, it is known to be antioxidant, antimicrobial, anti-inflammatory, analgesic, hepatoprotective, diuretic and antispasmodic (Herb Society of America 2005; Aprotosoaie et al. 2010).

Plants are known to contain several classes of bioactive compounds with antioxidant potential.

Chlorophylls (of which chlorophylls a and b are found in land plants) are the main pigments used in photosynthesis. They also have anti-inflammatory activity for consumers, enhance wound healing and inhibit calcium oxalate dihydrate accumulation (kidney stones), while limiting dietary uptake of some known carcinogens. Chlorophylls are among the main antioxidant compounds, preventing oxidative stress-associated diseases (Inanç 2011).

Carotenoids (including carotenes, lutein, lycopene, zeaxanthin) are accessory photosynthetic pigments. Some of them (especially carotene) are precursors of retinol and key to melanin synthesis, thus important for eye and skin functioning. They are also antioxidant and antiproliferative and researches show that lycopene is among the most important dietary anticarcinogens (Eldahshan & Singab 2013).

Phenolic compounds are a variate group of bioactive compounds, functioning as plant pigments, but also as antimicrobials and antifungals. Flavonoids (among them being anthocyanins – plant pigments and strong antioxidants), phenolic acids and tannins belong to this group (Kivrak & Kivrak 2014). For consumers, phenolic compounds are a key class of antioxidant, reducing and radical scavenging agents (Zymonė et al. 2018).

Ascorbic acid (vitamin C) is a key antioxidant, countering lipid oxidation at cell level in all organisms (Riscahyani et al. 2019)

Essential oils are complex mixtures of organic compounds, of which over 90% are volatile. Their composition includes terpenes (mostly mono- and sesquiterpenes), hydrocarbons and derivatives, flavonoids, aldehydes, alcohols, esters, etc., with a volatile fraction over 90%. They give plant products their specific aroma, while also having antimicrobial and antioxidant properties (Orphanides et al. 2011).

The objective of this paper was to determine the amount of several compounds and compound classes belonging to these categories in fennel leaves.

MATERIAL AND METHODS

Fennel leaves were collected from the experimental farm of the “Ovidius” University of Constanța.

Chlorophylls and carotenoids were determined by 80% acetone extraction and spectrophotometric absorption reading (S106 WPA spectrophotometer) at 470, 647, 663 nm (Popoviciu et al. 2020). Concentrations were calculated according to Lichtenthaler & Buschmann 2001.

Among individual carotenoid compounds, β-carotene, lycopene, lutein (and lutein esters), zeaxanthin and cryptoxanthin were determined by extraction in acetone:hexane:petroleum ether, petroleum ether, ethanol, acetone and using the spectrophotometric methods of Braniša et al. 2014, Sujith et al. 2010, Butnariu et al. 2014, Biehler et al. 2010, respectively.

The total concentration of phenolic compounds was determined by extracting plant tissue in methanol and reaction with Folin-Ciocâlteu reagent (10%) and sodium bicarbonate (7.5%) for 30 minutes. Absorbance was read at 765 nm against gallic acid calibration curve (Popoviciu et al. 2020). Flavonoids were determined by precipitation with hydrochloric acid and formaldehyde, followed by quantification of non-flavonoid phenolic content by Folin-Ciocâlteu reaction (de Lima et al. 2011). Anthocyanins were determined by 70% ethanol extraction and spectrophotometry at 520 and 700 nm (Braniša et al. 2014).

For total ascorbic and dehydroascorbic acid, ethanol extraction, reaction with ammonium molybdate and sulfuric acid, followed by spectrophotometric reading at 494 nm were employed (Riscahyani et al. 2019)

Essential oils were determined by petroleum ether extraction and gravimetry (Orphanides et al. 2011).

Dry biomass (23.25% on average) was determined through oven drying and used to express the concentrations of bioactive compounds in mg/kg DW.

RESULTS AND DISCUSSIONS

The concentrations of various classes of compounds with bioactive potential are shown in Figures 1-4.

Total chlorophyll content was 1,459 mg/kg, of which chlorophyll a was dominant (over 86%).

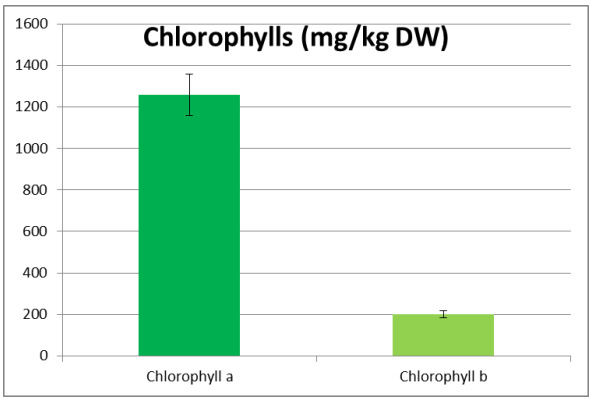

Figure 1. Concentrations of chlorophylls a and b in fennel leaves (mg/kg DW)

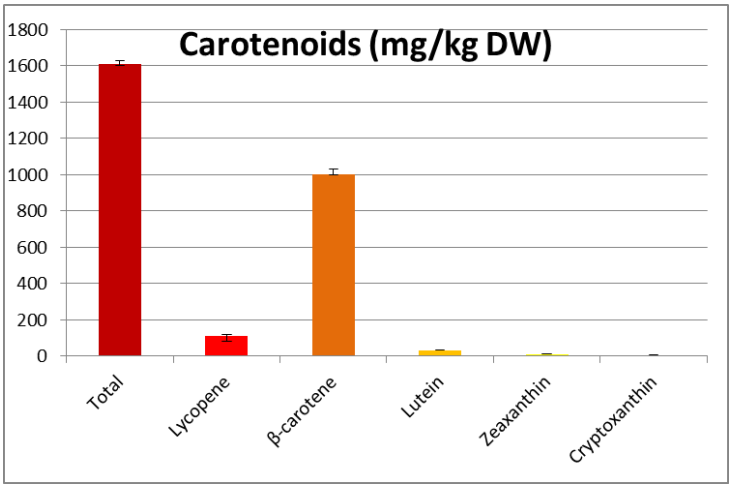

Figure 2. Concentrations of total and some individual carotenoids in fennel leaves (mg/kg DW).

Carotenoids were 1,609 mg/kg. Of this amount, β-carotene constituted over 62% (1,002 mg/kg). Lycopene amounted for 109 mg/kg (6.80% of the total amount). Lutein and xanthins were found only in small amounts. Apiaceae leaves are known to be a rich source of carotenoids, of which β-carotene is usually dominant. Researches on anise, chervil, caraway and dill found amounts of 126-179 mg/kg FW of this compound, lower than the 233 mg/kg FW equivalent found in

fennel. However, all these species also contain significant amounts of lutein (9-18 mg/kg FW compared to 7.42) rather than lycopene (Giordano et al. 2022). On the other hand, it should be noted that carotenoid content is a highly variable character, that can show major differences among cultivars of the same species, as shown in coriander (from 15 to 103 mg/kg β-carotene; Priyadarshi & Naidu 2019).

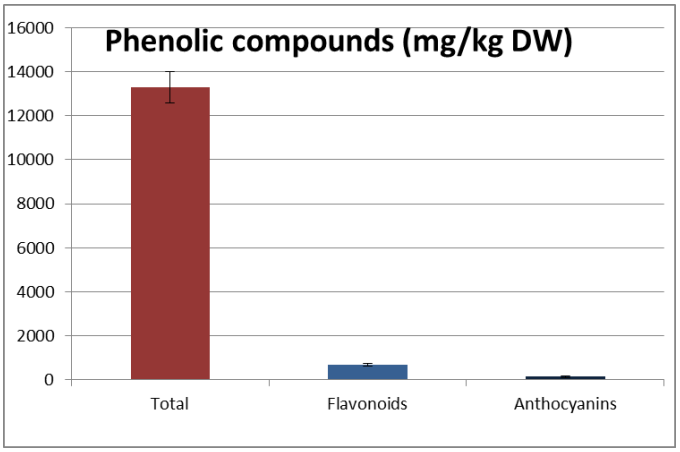

Figure 3. Concentrations of total phenolic compounds, flavonoids and anthocyanins in fennel leaves (mg/kg DW)

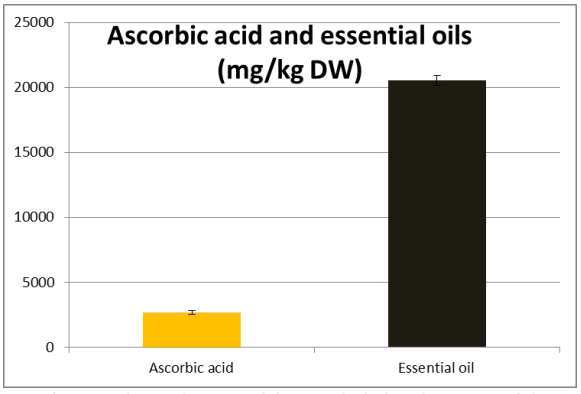

Figure 4. Concentrations of total ascorbic and dehydroascorbic acid and essential oils in fennel leaves (mg/kg DW)

Average total phenolic content was 13,292 mg/kg. Of these, the flavonoid fraction constituted only 5.16% (686 mg/kg). Of flavonoids, 21% (146 mg/kg) were anthocyanin pigments. For comparison, other phenolic contents found in Apiaceae leaves range from 2,300 (cumin) to 21,630 (parsley) mg/kg DW. However, in most cases flavonoids constitute 50% or higher (Deroiuch et al. 2020; Pricop et al. 2020; Thiviya et al. 2021).

2,658 mg/kg was the total amount of ascorbic and dehydroascorbic acid, while the average concentration of volatile oils was 20,553 mg/kg. The amount of

ascorbic acid (618 mg/kg FW), while high, is lower than those found in other Apiaceae, like chervil and dill (Giordano et al. 2022).

CONCLUSIONS

Fennel leaves had a high chlorophyll content (1,459 mg/kg DW), mostly chlorophyll a.

With 1,609 mg/kg DW, the carotenoid concentration was also high, dominated by β-carotene constituted over 62% (1,002 mg/kg). While lutein content was lower than in other related species, fennel leaves contained a high amount of lycopene (109 mg/kg DW).

At 13,292 mg/kg DW, the total phenolic inventory is comparable to that found in other Apiaceae. While rich in total phenols, fennel leaves had a low amount of flavonoids (686 mg/kg), of which 21% were anthocyanins.

Average ascorbic acid content was 2,658 mg/kg, comparable, but lower than in other Apiaceae species.

Fennel leaves were rich in essential oils, with an average concentration of 20,553 mg/kg DW.

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