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Coleostephus myconis, a Portuguese ruderal species with food purposes: fatty acids and tocopherols profile

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ABSTRACT

Coleostephus myconis (L.) Rchb.f. (Asteraceae) has ruderal and persistent growth. Considering its advantageous agronomic conditions, the phytochemical profile of this species should be comprehensively characterized before its potential use in food applications. In a previous study, we have characterized the antioxidant activity and phenolic composition of the green parts and flowers in three phenologic stages. Presently, the study was extended to the evaluation of the lipophilic compounds in the stems and leaves and senescent flowers, which respectively represent the botanical parts with the highest natural phytomass and those that allowed the best results regarding the phenolic composition and antioxidant activity. The senescent flowers were mostly characterized by their high contents in fat, oleic acid, β- and γ-tocopherol, while the stems and leaves stood out for their high contents in linolenic acid and α-tocopherol. In general, these results provide, for the first time, valid information regarding the application of *C. myconis* as food ingredient.

1. INTRODUCTION

Some researchers suggest that several world's plant species have medicinal value. Actually, wild plants are being increasingly studied and their natural components emerge as a strong alternative to synthetic ingredients, in a number of food products, due to their nutritional properties and bioactivity. Current research reports describe Asteraceae species as having immense potential to act as sources of bioactive compounds [1]. *C. myconis* (Asteraceae family) is a species with ruderal growth and persistence in abandoned soils. Their abundance and undervaluation constitute a research scope with great potential to be exploited in development of natural ingredients with food purposes. In our previous work, *C. myconis* was characterized for its phenolic profile and antioxidant activity [2]. Undoubtedly, the highest antioxidant activity was measured in the hydro-ethanolic extracts, particularly those obtained from the senescent flowers, probably due to their higher contents in phenolic compounds. In this perspective, senescent flowers and green parts (stems and leaves) of *C. myconis* were characterized regarding nutritional value, anticipating their potential application in food products. Particular attention was given to lipophilic compounds i) fatty acids composition: polyunsaturated (PUFA), monounsaturated fatty acids (MUFA) and unsaturated fatty acids/saturated fatty acids (SFA) ratio; ii) tocopherols isoforms profile. In fact, fatty acids were reported as having important health effects, *e.g.*, preventing cardiovascular diseases and minimizing the related risk factors, presenting anti-carcinogenic and anti-inflammatory activities, besides having neuroprotective activity and acting as growth promoters by contributing to bone development [3]. Vitamin E, on the other hand is mainly acknowledged for its high antioxidant activity, specifically in preventing lipid peroxidation. A number of studies showed that its requirements are related to the dietary intake of PUFA [4]. Thereby, it is highly relevant to evaluate the lipophilic compounds profile of new possible food ingredients. Despite the relative dissemination of scientific works reporting Asteraceae species, *C. myconis* is nearly absent from scientific literature.

2. MATERIALS AND METHODS

Total fat was determined by Soxhlet extraction with petroleum ether according to the AOAC methodology [5].

2.1 Fatty acids

Fatty acids were prepared, in triplicate, by trans-methylation using boron trifluoride (Sigma Aldrich St. Louis, MO, USA), based on the methodology developed by Shantha and Ackman [6]. Derivatization process was initiated by mixing 40 µL of the extracted oil, 3 mL of hexane and 200 µL of potassium hydroxide (2 M). After vortexing (1 min), 500 mg of Na_2SO_4 were added to eliminate any remaining water. This mixture was further vortexed and centrifuged (3000 rpm, 5 min, Heraeus Sepatech Labofugue Ae, Heraeus Instruments, Hanau, Germany). The supernatant was transferred to a vial and kept at -20 ºC, until further analysis [7].

The analysis was carried out with a Shimadzu GC-2010 gas chromatograph equipped with a split-splitless injector, a flame ionization detector (FID) and a Shimadzu AOC-20i autosampler (Shimadzu, Tokyo, Japan). The chromatograph was equipped with a CPSil 88 fused silica capillary column (Varian, Middelburg, Netherlands; 50 m × 0.25 mm i.d., 0.19 µm film thickness). Fatty acid methyl esters (FAME) were identified by comparing their relative retention times with a standard mixture (FAME 37, Supelco, Bellefonte, PA, USA) and analyzed using the Shimadzu software GC Solution (v. 2.30, Shimadzu GC Solution, Shimadzu, Tokyo, Japan) based on the relative peak areas. The results were expressed in relative percentage of each fatty acid.

2.2 Tocopherols

Standard solutions of α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol were prepared in n-hexane (25, 18.75, 12.5, 6.25, 2.5 and 1.25 mg/mL). Each of these solutions contained 20 µL of tocol (internal standard, 1 mg/mL). The chromatographic analysis was carried out in an HPLC integrated system equipped with an AS-2057 automated injector, a PU-2089 pump, a MD-2018 multiwavelength diode array detector and a FP-2020 fluorescence detector (Jasco, Tokyo, Japan), programmed for excitation at 290 and emission at 330 nm [8]. The chromatographic separation was achieved on a normal phase Supelcosil™ LC-SI column. Data were analyzed using JASCO-Chrom NAV Chromatography Software. The compounds were identified based on their UV/vis spectra and the respective patterns retention time. Quantification was based on the fluorescence signal response of each standard, converted to concentration units through calibration curves obtained from commercial standards of each compound, using the internal standard method. The results were expressed in μg/g of fat.

3. RESULTS AND DISCUSSION

Fat contents varied among 1.4 g/100 g dm, in the green parts, and 3.6 g/100 g dm in the senescent flowers. The main fatty acids (FA) found in senescent flowers were linoleic acid (C18:2n6c) (43%), palmitic acid (C16:0) (21%) and oleic acid (C18:1n9c) (15.3%), while in stems and leaves the most abundant forms were linolenic acid (C18:3n3) (51%), followed by C18:2n6c (26%) and C16:0 (16%) (**Table 1**).

Table 7. Fatty acids profile (relative %) in stems and leaves and senescent flowers of *C. myconis*.

Fatty acid	Stems and leaves	Senescent flowers	*t*-Student test *p* value
C10:0	nd	2.4±0.1	-
C12:0	nd	0.66±0.05	-
C14:0	0.39±0.03	1.8±0.1	<0.001
C15:0	0.13±0.01	0.18±0.01	<0.001
C16:0	16±1	21±1	<0.001
C16:1	1.00±0.03	nd	-
C18:0	1.5±0.1	4.5±0.2	<0.001
C18:1n9c	4.5±0.5	15.3±0.4	<0.001
C18:2n6c	26±1	43±1	<0.001
C18:3n3	51±1	9.3±0.3	<0.001
C20:0	nd	0.46±0.01	-
C22:0	nd	0.77±0.05	-
C23:0	nd	0.54±0.05	-
C20:5n3	nd	0.54±0.05	-
SFA	18±1	32±2	<0.001
MUFA	5±1	15±1	<0.001
PUFA	77±2	53±1	<0.001

nd: non detected

Polyunsaturated fatty acids (PUFA) predominated in both C. myconis botanical parts (53% of total FA in senescent flowers; 77% in stems and leaves). Monounsaturated fatty acids (MUFA) are present in less relevant percentages: 15% in senescent flowers and 5% in stems and leaves. However, the unsaturated fatty acids/saturated fatty acids (SFA) ratio scored 2.1 for senescent flowers and 4.6 for stems and leaves, increasing the nutritional potential value of *C. myconis* as a food ingredient, which is also enhanced by the predominance of omega-3 and omega-6 fatty acids.

In fact, these fatty acids were reported as having important health effects, *e.g.*, lowers blood cholesterol and LDL cholesterol concentrations, preventing cardiovascular diseases and minimizing the related risk factors. Additionally, it is now clear that they influence a range of other diseases, including metabolic diseases such as type 2 diabetes, inflammatory diseases such as arthritis, and cancer [3,9].

Regarding vitamin E profile, α- (74 µg/g oil), β- (99 µg/g oil), γ- (37 µg/g oil) and δ-tocopherol (91 µg/g oil) were detected in the senescent flowers, while stems and leaves presented only α- and δ-tocopherol, in both cases in higher amounts (α-tocopherol: 110 µg/g oil; δ-tocopherol 215 µg/g oil) (**Table 2**). Despite representing a small percentage of the DDR for vitamin E (10-15 mg/day), the detected levels might be considered as relevant, especially owing their disease preventing effects, which are often related to their high antioxidant activity. In atherosclerosis and related diseases, for instance, tocopherols exert their effect through inhibiting the lipid peroxidation and platelet aggregation, while reducing the anti-inflammatory response [4].

Table 8. Tocopherols profile (µg/g oil) in stems and leaves and senescent flowers of *C. myconis*.

Vitamer	Stems and leaves	Senescent flowers	*t*-Student test *p* value
α-Tocopherol	110±14	74±11	<0.001
β-Tocopherol	nd	99±10	-
γ-Tocopherol	nd	37±10	-
δ-Tocopherol	215±5	91±14	<0.001
Tocopherols	325±17	165±22	<0.001

nd: non detected.

4. CONCLUSION

Considering lipophilic compounds (fatty acids, tocopherols) profile, *C. myconis* can be considered as a wild plant with high interest for incorporation in functional foods or food supplements. The assayed botanical parts exhibited significant differences among the assayed parameters, thereby raising the possibility of being used in complementary applications.

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