Phenolic acids in *Crithmum maritimum* L. (Apiaceae) after Tytanit fertilization

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Abstract

Aerial parts and roots of *Crithmum maritimum* L. fertilized with Tytanit were investigated on the presence of phenolic acids (PhAs). Cinnamic and benzoic acid derivatives were quantified by use of validated RP-HPLC/DAD method. The amount of PhAs in fertilized plants (T) was higher than in control (C) plants (in the aerial parts: 2.16 mg/g and 1.28 mg/g dry weight, respectively, and in roots: 4.05 mg/g and 2.78 mg/g dry weight, respectively). The predominant PhA was the caffeic acid (83.2–94.2% of the total PhAs). After Tytanit treatment, amount of the caffeic acid rose from 667.41 µg/g in C to 1463.83 µg/g dry weight in the aerial parts of T, and in roots from 2251.74 µg/g in C to 3451.86 µg/g dry weight in T. Tytanit had also influence on the qualitative composition of PhAs; in extracts from aerial parts, some of PhAs (ferulic, chlorogenic, and syringic acids), absent in control, appeared after fertilization.

Keywords

phenolic acids; RP-HPLC/DAD; *Crithmum maritimum*; Tytanit; caffeic acid

Introduction

*Crithmum maritimum* L., sea fennel, rock samphire (family Apiaceae), is a perennial, halophyte (resistant to salinity), strong aromatic species found in the wild on maritime rocks, cliffs, and sands along the Atlantic, Mediterranean, and Black Sea coasts [1: p. 333; 2: p. 367, 3–5]. In past times, sailors used to consume leaves of *C. maritimum* to prevent scurvy [6]. Nowadays, leaves of this plant are consumed fresh and pickled [7,8]. An infusion of leaves, which have a fennel-like aroma and taste, tonic and depurative properties, is said to promote digestion and diuresis [8,9]. Also, insecticidal and antimicrobial properties of *C. maritimum* were confirmed [10–12]. Vitamins [7,13], minerals, flavonoids and catechins [3], proteins and amino acids [14,15], lipids and fatty acids [16], coumarins [17], acetylenes [18], essential oil constituents [19–22] in a sea fennel were detected before. Recently, content of flavonoids, tannins, and also total polyphenols in the different parts of *C. maritimum* was evaluated and the presence of phenolic acids (PhAs), i.e., benzoic and cinnamic acid derivatives, qualitatively analyzed by HPLC [3,23]. In vitro digestion model of antioxidant capacity of aqueous infusions from wild-grown *C. maritimum* was studied and chlorogenic acid and its derivatives were the most important class of polyphenols found in those extracts [24]. Several plants containing PhAs are widely used in phytotherapy. Various pharmacological properties of PhAs, e.g., antioxidant, antiviral, and cholesterol-reducing activity, make them potentially protective against cancer and coronary heart diseases [25–27]. PhAs inhibit angiotensin-converting enzyme and play important role in hypertension prevention [28]. They have also antimicrobial and antiviral properties [29] and, as components of the human diet in combination with antitumor drugs, can reduce side effects of...
the anticancer therapy [30–32]. PhAs regulate tumor promoting metabolic pathways and can inhibit tumor development [33,34]. In plants, PhAs play role as phytoalexins and also perform defensive functions against pathogenic agents. [35]. Evaluation of phenolic acids in plants is therefore important from both phytotherapeutical and plant physiology perspective.

Titanium is one of the so-called “beneficial elements” for plants, i.e., chemical elements that improve the organism’s health status, but the organism can grow and healthily develop in their absence [36]. The titanium influence on plants has been investigated for almost 100 years [37,38]. As cited by Tlustoš et al. [39], in 1913, Traetta-Mosca observed that Ti enhanced the growth of tobacco plants, and it was found by Geilmann in 1920 that Ti accumulates mainly in assimilation organs. First systematic research concerning Ti effect on plants was done by Nemec and Kas in 1923 [40]. Authors observed Ti dose-response relationship; in some “optimal” concentration of Ti ions, plants became greener and growth and development of plants was intensified (chlorophyll content and leaf area was increased). However, at higher dose of Ti, the effect was opposite. These results were later confirmed by Hrubý et al. [41] in hydroponic experiments and by Radkowski [42] by experiments in the agricultural soil assigned to class I, wheat group of very good quality. Titanium feeding makes plants less vulnerable to adverse environmental conditions, improves their resistance against bacterial and fungal diseases [43]. The use of Ti significantly improves the health status of the plants and increases crops yields at a very low concentration, which was found to be non-toxic for animals and for humans [37,38].

Numerous theories on the mechanism of Ti action in plants were proposed and many of them are summarized in the reviews by Carvajal and Alcaraz [38] or by Hrubý et al. [41]. It was suggested that the biological effects of Ti were based upon the defense mechanisms of the plant organism against Ti; the low dose increases defense mechanisms and large (toxic) dose decreases them (hormesis effect) [41]. Because naturally occurring Ti is found in the form of minerals insoluble in water (e.g., TiO₂ or FeTiO₃), it is unavailable to plants [44] and must be supplied in soluble form. Pais [36,37] found Ti(IV) ascorbate to be water-soluble compound which is pH stable (up to pH 8) and not toxic for the living system [38]. The enhancement of the biological functions of plants was induced by the cation Ti⁴⁺, whereas the anion (ascorbate) did not have any effect [45]. Successful experiments with Tytanit, the mono-component fertilizer which contains Ti(IV) ascorbate as an active compound, were performed previously [42,46,47]. In C. maritimum fertilized with Tytanit, the effect on qualitative and quantitative composition of the essential oil in the aerial parts was analyzed [48]. In the present study, in one-factor experiment, we analyzed for the first time the influence of the Tytanit on the composition of PhAs in C. maritimum L. aerial parts and roots.

Material and methods

Standards and chemicals

Phenolic acid standards (purity ≥98%, HPLC assay, Sigma-Aldrich, USA), methanol and acetic acid, chromatography grade (J. T. Baker Inc., the Netherlands), an ultrapure water (18.2 mΩ) from a Simplicity Millipore purification system (Molsheim, France), were used. All solvents were degassed in an ultrasonic bath. The other solvents and reagents were of analytical grade (POCH, Poland). Tytanit (Intermag, Poland), the mono-component fertilizer, contains Ti⁴⁺ ions in amount of 8.5 g/L as Ti(IV) ascorbate.

Plant material

Crithmum maritimum L. (Apiaceae) was collected in the garden of Medical University in Gdańsk, Poland (where the voucher specimen was deposited) and was identified by KWR. The experiment was performed on an agricultural soil. One-year-old plants were cultivated from the same pool of seeds. Plant laboratory samples were prepared in accordance with European Pharmacopoeia (sixth edition) guidelines [49].
Fertilization with Ti⁴⁺ ions

From two equivalent groups of cultivated plants, one was fertilized by spraying the leaf surface with 0.04% Tytanit water solution (T), the second, control group (C), was sprayed only with water (each time 200 mL per plant). Fertilization started when first leaves appeared, followed by every 7 days until the flowering starts. Plants were harvested 7 days after last fertilization, washed with deionized water, and dried (3 days at 40°C). Aerial parts (1) and roots (2) of the plants (T and C), were ground and analyzed. The amount of phenolic acids was expressed as dry weight (DW) of the plant material.

Extraction of the plant material

Dried plant material (1/T and 1/C – aerial parts, 2/T and 2/C – roots; 10 g of each) macerated (24 h) and extracted – defatted (24 h) in Soxhlet apparatus with petroleum ether (200 mL) was next macerated (24 h) and extracted with methanol (200 mL at 70°C). Free PhAs fractions were obtained as described previously by Ibrahim and Towers [50]. The methanol extracts, evaporated to dryness and dissolved in 100 mL of hot water, were left in a refrigerator for 24 h, filtered (quantitative filter paper type 388, Whatman, UK), and water fraction (W1) was collected. W1 was extracted with diethyl ether (5 × 20 mL), next washed with 5% aqueous sodium bicarbonate solution (5 × 20 mL), acidified, extracted with diethyl ether (5 × 20 mL), dried over anhydrous sodium sulfate, and evaporated to dryness. Residue, dissolved in methanol, contained fraction with free phenolic acids. Water fraction W2, ca. 100 mL, obtained as a waste, was subjected to further hydrolyses.

Alkaline hydrolysis of esters and acid hydrolysis of glycosides

Acid and alkaline hydrolyses were carried out in accordance with the procedure described by Schmidtlein and Herrmann [51]. For this purpose, water fraction W2 was divided into two parts (50 mL each) and used for acid and alkaline hydrolyses. Acid hydrolysis was performed in 36% HCl (pH 1.5) and alkaline hydrolysis with Ba(OH)₂ (pH 12) with a reductive medium containing NaBH₄ (E. Merck, Germany) for 30 min at 100°C. Fractions after alkaline hydrolysis were acidified with 10% H₂SO₄ (to pH 1.5). Both fractions were extracted with diethyl ether (5 × 20 mL), dried over anhydrous sodium sulfate, evaporated to dryness, and dissolved in methanol.

Sample clean-up

Obtained fractions were evaporated to dryness under vacuum (50°C), dissolved in 5 mL of 50 aqueous methanol, and passed through 0.45 µm PTFE membrane filters (Whatman, UK). The following samples were obtained: C-F and T-F – PhAs-free fractions; C-AH and T-AH – PhAs released after acid hydrolysis; C-BH and T-BH – PhAs released after alkaline hydrolysis.

RP-HPLC analysis of individual compounds and UV spectroscopy

The chromatographic system consisted of Hewlett-Packard (USA) HP 1100 liquid chromatograph equipped with an AG 1315A UV-visible diode array detector (DAD) and Hypersil BDS C18 (250 mm × 4.6 mm I.D., 5 µm) column (Agilent Technologies, UK), and controlled by HP ChemStation rev. 10.0 software (Agilent). The reversed-phase high performance liquid chromatography (RP-HPLC) was performed according to a procedure described elsewhere [52]. Hypersil ODS C18 (250 mm × 4.6 mm I.D., 5 µm) column (Agilent Technologies) was used as the stationary phase. Methanol–water (20:80) with 1% acetic acid (v/v, temp. 25°C) was the mobile phase. UV spectra (λ = 200–400 nm) of compounds were acquired on-line. Ten-µL samples were injected. Detection was performed at 254, 280, and 320 nm. The selectivity of the method and identification
of the compounds were achieved by comparison of $t_R$ values and UV-DAD spectra of compounds in analyzed samples and standards under the same chromatographic conditions. For quantitative determination, calibration curves for standards were prepared at 280 nm, except for cinnamic acid derivative, caffeic acid (at 320 nm), and benzoic acid derivative, vanillic acid (at 254 nm). The integration was done manually. Stock standard solutions were prepared by dissolving PhAs (1 mg of each) in 10 mL of 50% aqueous methanol (J. T. Baker). For statistical evaluation Student’s $t$ test was used ($p = 95\%$, $\alpha = 0.05$).

**Method validation**

Method for quantitative evaluation of PhAs followed ICH guidelines [53,54] and was validated with respect to linearity, selectivity, sensitivity, accuracy (tested as recovery), precision (intra- and interday), and limit of detection (LOD) and limit of quantification (LOQ). Injection repeatability was validated by injecting aliquots of the same sample six times during a day, and the precision was validated by determination of relative standard deviation (%) of peak areas of six aliquots in the same day (in intraday tests) and on three consecutive working days (in interday tests). The calibration plots for PhAs ($n = 3$, at five concentrations) were characterized by their regression coefficients, and the slope (a) and intercept (b) form for the regression equation of $y = ax + b$. LOD and LOQ for each PhA were calculated as follows; $LOD = 3\sigma a^{-1}$ and $LOQ = 10\sigma a^{-1}$, where $\sigma$ is the standard deviation of the response and $a$ is the slope of the calibration curve. On the basis of quantitative results, the percentage composition of PhAs was determined by assuming that the total amount (in µg/g DW) of the detected PhAs in each fraction constituted 100%.

**Results**

**Method validation**

Linear parameters for PhAs standards (regression equations, LOD, and LOQ) and retention times of PhAs from the analyzed samples in applied RP-HPLC method are listed in the Tab. 1.

RSD measured as injection repeatability was ≤2.6% and RSD as a measure of intraday and interday precision was in each case less than 5%. Accuracy measured as recovery for protocatechuic acid in samples 1/C-F, 1/C-AH, and 1/C-BH at three fortification levels (10, 25, and 50 mg/100 mL; $n = 3$) to water fractions (W1 and W2, respectively) showed (for applied multistep extraction procedures) satisfactory results, from 85.5% (1/C-BH samples) to 92.3% (1/C-F samples). The calibration plots for all the standards were linear ($R^2 \geq 0.9996$) in the concentration range 0.01–0.2 mg mL$^{-1}$ for each PhAs, except for caffeic acid where concentration range was 0.02–1.0 mg/mL$^{-1}$.

**Analysis of the samples**

In the analyzed C. maritimum L. extracts by RP-HPLC/DAD, the benzoic acid derivatives, namely, protocatechuic, gentisic, $p$-hydroxybenzoic, vanillic, and syringic acids and the cinnamic acid derivatives, i.e., caffeic, $p$-coumaric, and ferulic acids, and the depside – chlorogenic acid, were identified and quantified. Fig. 1 presents HPLC chromatogram of an extract from roots (2/T-AH) determined at 280 nm. As we could observe, vanillic and caffeic acids were not separated to the baseline and therefore quantification was done for vanillic acid at 254 nm (at this wavelength, the peak of caffeic acid was not observed) and for caffeic acid at 320 nm (at this wavelength, the peak of vanillic acid was absent).

Fertilization with Tytanit had an influence on the amount of PhAs present in extracts in ester form (released after alkaline hydrolysis) and, interestingly, only a weak influence on the amount of the free PhAs and those released after acid hydrolysis. The total amount
of PhAs (HPLC/DAD-assay) in extracts from fertilized plants was higher compared to
the control, 2.16 mg/g and 1.28 mg/g of DW in the aerial parts, respectively, and 4.05
mg/g and 2.78 mg/g of DW in roots, respectively (Tab. 2, Tab. 3).

After fertilization with Titanit formula, the amount of the caffeic acid rose significantly,
from 667.41 µg/g in control sample (1/C-BH) to 1463.83 µg/g DW in the aerial parts
of fertilized plants (1/T-BH) (Tab. 2), and in roots from 2251.74 µg/g in control sample
(2/C-BH) to 3451.86 µg/g DW in fertilized plants (2/T-BH) (Tab. 3), which was ca. 52%
higher. In extracts from aerial parts, some of PhAs appeared after fertilization – ferulic
acid in fraction of free phenolic acids (1/T-F), chlorogenic acid in fraction after acid

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**Tab. 1** Linear parameters for PhAs standards (regression equations, LOD, and LOQ) and retention times of PhAs from the analyzed samples in applied RP-HPLC method.

<table>
<thead>
<tr>
<th>No.</th>
<th>Phenolic acid</th>
<th>Selectivity and sensitivity</th>
<th>tR values in the chromatographic system tested (n = 6)</th>
<th>LOD  (µg/mL)</th>
<th>LOQ  (µg/mL)</th>
<th>Regression line (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protocatechuic</td>
<td></td>
<td>5.776 ± 0.024</td>
<td>0.045</td>
<td>0.137</td>
<td>y = 14.764x – 44.35</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic</td>
<td></td>
<td>8.390 ± 0.032</td>
<td>0.065</td>
<td>0.195</td>
<td>y = 20.006x – 6.32</td>
</tr>
<tr>
<td>3</td>
<td>Gentisic</td>
<td></td>
<td>8.522 ± 0.020</td>
<td>0.024</td>
<td>0.068</td>
<td>y = 26.712x – 87.12</td>
</tr>
<tr>
<td>4</td>
<td>p-Hydroxybenzoic</td>
<td></td>
<td>9.592 ± 0.021</td>
<td>0.037</td>
<td>0.113</td>
<td>y = 16.173x – 52.08</td>
</tr>
<tr>
<td>5</td>
<td>Vanillic</td>
<td></td>
<td>11.887 ± 0.030</td>
<td>0.036</td>
<td>0.111</td>
<td>y = 14.766x – 32.52</td>
</tr>
<tr>
<td>6</td>
<td>Caffeic</td>
<td></td>
<td>12.363 ± 0.053</td>
<td>0.020</td>
<td>0.062</td>
<td>y = 9.931x – 107.34</td>
</tr>
<tr>
<td>7</td>
<td>Syringic</td>
<td></td>
<td>13.985 ± 0.034</td>
<td>0.010</td>
<td>0.029</td>
<td>y = 26.562x – 90.86</td>
</tr>
<tr>
<td>8</td>
<td>p-Coumaric</td>
<td></td>
<td>23.217 ± 0.026</td>
<td>0.021</td>
<td>0.064</td>
<td>y = 28.724x – 97.18</td>
</tr>
<tr>
<td>9</td>
<td>Ferulic</td>
<td></td>
<td>29.531 ± 0.038</td>
<td>0.007</td>
<td>0.020</td>
<td>y = 81.267x – 33.71</td>
</tr>
</tbody>
</table>

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Fig. 1  RP-HPLC chromatogram (280 nm) of AH-T fraction from *C. maritimum* roots. Stationary phase: Hypersil ODS C18 (250 mm × 4.6 mm I.D., 5 µm); mobile phase: methanol–water (20:80) with 1% acetic acid (2/T-AH; acid hydrolysis after treatment with Titanit). PhAs: protocatechuic (1), chlorogenic (2), vanillic (5), caffeic (6), syringic (7), p-coumaric (8), ferulic (9) acid; X – unidentified, with syringic acid-like DAD spectra.
Tab. 2  The content of phenolic acids in aerial parts of *Crithmum maritimum* L. plants fertilized with Tytanit, compared with control plants.

<table>
<thead>
<tr>
<th>No.</th>
<th>Phenolic acid</th>
<th>Mean ±SD (µg/g DW) n = 4</th>
<th>control (C)</th>
<th>Tytanit (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/C-F</td>
<td>1/C-AH</td>
</tr>
<tr>
<td>1</td>
<td>Protocatechuic</td>
<td>27.18 ±0.62</td>
<td>26.44 ±0.15</td>
<td>26.85 ±2.96</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>3</td>
<td>Gentisic</td>
<td>3.55 ±1.06</td>
<td>5.92 ±0.40</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>p-Hydroxybenzoic</td>
<td>9.41 ±0.60</td>
<td>nd</td>
<td>22.97 ±0.85</td>
</tr>
<tr>
<td>5</td>
<td>Vanillic</td>
<td>15.20 ±0.36</td>
<td>13.32 ±0.02</td>
<td>39.78 ±1.38</td>
</tr>
<tr>
<td>6</td>
<td>Caffeic</td>
<td>63.82 ±1.80</td>
<td>152.76 ±4.62</td>
<td>667.41 ±30.03</td>
</tr>
<tr>
<td>7</td>
<td>Syringic</td>
<td>nd</td>
<td>15.88 ±0.11</td>
<td>nd</td>
</tr>
<tr>
<td>8</td>
<td>p-Coumaric</td>
<td>7.60 ±0.24</td>
<td>13.70 ±0.10</td>
<td>38.60 ±1.36</td>
</tr>
<tr>
<td>9</td>
<td>Ferulic</td>
<td>nd</td>
<td>20.32 ±0.25</td>
<td>104.88 ±6.64</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>126.76 ±2.68</td>
<td>248.36 ±4.77</td>
<td>900.96 ±38.37</td>
</tr>
</tbody>
</table>

F – free phenolic acids; AH – phenolic acids released after acid hydrolysis; BH – phenolic acids released after alkaline hydrolysis; nd – not detected; Anova: n = 4, α = 0.05, p = 95%.
Tab. 3 The content of phenolic acids in roots of *Crithmum maritimum* L. plants fertilized with Tytanit, compared with control plants.

<table>
<thead>
<tr>
<th>No.</th>
<th>Phenolic acid</th>
<th>Mean ± SD (µg/g DW) n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>control (C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/C-F</td>
</tr>
<tr>
<td>1</td>
<td>Protocatechuic</td>
<td>8.29 ±0.29</td>
</tr>
<tr>
<td>2</td>
<td>Chlorogenic</td>
<td>nd</td>
</tr>
<tr>
<td>3</td>
<td>Gentisic</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>p-Hydroxybenzoic</td>
<td>7.22 ±0.15</td>
</tr>
<tr>
<td>5</td>
<td>Vanillic</td>
<td>15.36 ±0.92</td>
</tr>
<tr>
<td>6</td>
<td>Caffeic</td>
<td>85.98 ±1.70</td>
</tr>
<tr>
<td>7</td>
<td>Syringic</td>
<td>8.97 ±0.04</td>
</tr>
<tr>
<td>8</td>
<td>p-Coumaric</td>
<td>8.18 ±0.24</td>
</tr>
<tr>
<td>9</td>
<td>Ferulic</td>
<td>2.90 ±0.23</td>
</tr>
<tr>
<td>X</td>
<td>Calculated as syringic acid</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>136.91 ±3.29</td>
</tr>
</tbody>
</table>

F – free phenolic acids; AH – phenolic acids released after acid hydrolysis; BH – phenolic acids released after alkaline hydrolysis; X – unindentified; tr – traces (under quantification limit); nd – not detected; Anova: n = 4, α = 0.05, p = 95%.
hydrolysis (1/T-AH), and syringic acid in fraction after alkaline hydrolysis (1/T-BH). Gentisic acid present in plant control as a free phenolic acid (1/-C-F) was absent in free phenolic acid fraction from fertilized plants (1/T-F). In roots, p-hydroxybenzoic acid, present only in traces in fraction after alkaline hydrolysis from control plants (2/C-BH), rose to 15.01 µg/g DW in 2/T-BH plant extracts (Tab. 2, Tab. 3). The caffeic acid was the predominant phenolic acid in all investigated extracts in the aerial parts and accounted for: 49.6–50.4% (1/T-F), 61.5–61.4% (1/T-AH), 74.1–83.2% (1/T-BH), and in the roots: 62.8–64.3% (2/T-F), 60.8–58.7% (2/T-AH), 93.5–94.2% (2/T-BH).

Discussion

In a study by Maleš et al. [3], total polyphenol content in aerial parts of a sea fennel was relatively high (4.72–9.48%). As found (qualitatively) by Jallali et al. [23], free PhAs such as gallic, caffeic, chlorogenic, vanillic, rosmarinic, p-coumaric, cinnamic and 2-hydroxy cinnamic acids were identified among other polyphenols (flavonoids and catechins) in C. maritimum from the natural source. Polyphenols were quantified by use of colorimetric method with Folin–Ciocalteu reagent and calculated as an equivalent of a gallic acid. Meot-Duros and Magné [55] and also Jallali et al. [23] reported that antioxidant activities of extracts from C. maritimum are related to their phenolic composition. The polyphenolic content in C. maritimum varied in accordance to the phenological stage of the plant [23], increasing at the start of the flowering period; at this stage plants were harvested in our study. Extraction technique has a big influence on the phenolic content and composition. Soxhlet extraction, which was reported as an efficient method for obtaining PhAs from plants [56,57], was used in our experiments. As expressed by many authors [58,59], composition of the phenolics, and among them also phenolic acids, depends on both environmental conditions (temperature, humidity, exposure to light) and biological influences (e.g., insects and herbivores) [60]. Tytanit and its active component, Ti$^{4+}$ ions, were investigated previously, and their effect on biomass production and condition of plants was confirmed [42,46–48]. In our study, we present the influence of Ti$^{4+}$ ions on the production of PhAs. Titanium feeding, by its addition to the soil or by spraying onto the leaf surface, was previously investigated and discussed [42,61,62]. Hrubý et al. [41] revealed low Ti mobility within a plant when only roots were treated. Effects of Ti ions action when applied to leaves were also detected in fruits, resulting in fruits ripening [61]. Further enhancement of biological functions in Capsicum annuum was observed when Ti ions were sprayed on leaves [43], therefore we decided to use this approach in our experiments. Siracusa et al. [24] tested infusions from C. maritimum in gastrointestinal model. They found out that total polyphenol content and its antioxidant capacity decreased in digested samples. Therefore, it might be worth considering to stimulate production of polyphenols in plants used as antioxidants source. It could enhance therapeutic potential of treated plants and would be of big importance from the phytotherapeutical perspective, because, as was mentioned previously, C. maritimum is a promising source of phenolic bioactive compounds.

In conclusion, it is worth to stress that fertilization with Tytanit increases total content of PhAs in treated C. maritimum plants, especially the amount of caffeic acid bonded as esters.

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