

## Article

# Organic and Conventional Herbs Quality Reflected by Their Antioxidant Compounds Concentration

Ewelina Hallmann \*  and Piotr Sabała

Department of Functional and Organic Food, Institute of Human Nutrition Sciences,  
Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland; pio.sab@wp.pl

\* Correspondence: ewelina\_hallmann@sggw.pl; Tel.: +48-22-59-370-36

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**Abstract:** The aim of this work was to determine the bioactive compounds concentration in herbs from organic and conventional production. In 2017 and 2018, herbs of four species, including basil (*Ocimum basilicum* L.), bear's garlic (*Allium ursinum* L.), marjoram (*Origanum majorana* L.), and oregano (*Origanum vulgare* L.), were examined. The concentrations of polyphenols, phenolic acids, flavonoids, carotenoids, and chlorophylls were measured. Next, separation and identification of the individual bioactive compounds were completed. The obtained results show that organic herbs contained significantly higher concentrations of total polyphenols, flavonoids, and phenolic acids compared to conventional herbs in both investigation years. On the other hand, conventional herbs contained significantly higher concentrations of chlorophylls and carotenoids, including beta-carotene.

**Keywords:** organic herbs; conventional herbs; polyphenols; flavonoids; carotenoids; chlorophylls

## 1. Introduction

Herbs and spices stimulate the appetite and positively influence the function of the human body by enhancing the digestion and absorption of food [1]. They can also increase the shelf life of food [2–4]. Herbs and spices enrich the sensory experience and are an important element in food preparation [5]. In Poland, interest in cultivating and using herbs has been growing in recent years. Poland is not a leading producer of organic herbs, spices, and aromatic plants, producing 3.24 tons per year. The total area of this production is 6.95 thousand ha [6]. Compared to conventional production, total organic production is relatively low (in conventional systems, the crop area was 103.3 thousand ha and the annually production was 34.4 thousand tons). However, the production of organic herbs and spices is systematically growing. Poland ranks fourth in Europe in terms of organic herbs production. Among the European countries, the largest producer of organic herbs is Turkey, with 54.0 thousand tons in an area of 49.7 thousand ha. Consumer interest in organic herbs is growing in recent years [7]. Organic herb production is associated with strict rules [8]. In organic systems, use of synthetic plant protection products (pesticides) and fertilizers is prohibited. For fertilization, organic fertilizers (manure and compost) are widely used. In an area of plant protection, only natural methods can be used, such as sticky boards, pheromone traps, and plant extracts [9]. Spices and herbs contain numerous biologically active compounds from the groups of polyphenols and carotenoids. These compounds are called “natural pesticides” and are used by plants against pests [10]. These compounds have strong antibacterial, antioxidant, anticancer, and pro-health effects [11–13]. Many studies indicate that organic herbs and aromatic plants are characterized by a higher concentration of biologically active compounds [14–16]. In shops, many organic herbs and spices are found; their quality is obviously dependent on the species, method of cultivation, method of processing, or even the manufacturer. Knowledge about the quality of organic herbs and the content of biologically active compounds is

still insufficient. The aim of the work was to show the effect of production system (organic and conventional) on the bioactive compounds concentration in herbs.

## 2. Materials and Methods

For study purposes, herbs of four plant species were chosen: basil (*Ocimum basilicum* L.), bear's garlic (*Allium ursinum* L.), marjoram (*Origanum majorana* L.), and oregano (*Origanum vulgare* L.). These are some of the most popular herbs in Polish market. In 2017 and 2018, herb samples were purchased from organic or conventional producers, the two largest producers on Polish market. All organic herbs were certified (PL-EKO-01). In both years, the samples came from two different production batches and were purchased and analyzed in February. According to the information from the producers (both organic and conventional), the herbs sold in 2017 and 2018 were harvested in 2016 and 2017, respectively. After the drying process, samples of herbs were packaged in plastic bags and paper boxes (organic) as well as in paper bags with aluminum foil (conventional) and kept in a special storage room (temperature 15 °C) for a duration of four months by producers. At the beginning of February (both years), herbs were brought to the shops. Therefore, the choice of material for the present investigation could be identified as a "basket study". This kind of study is very important from the consumer point of view. It shows the diversification of herbs in Polish stores and their quality. Each of the studied herbs from conventional and organic production in the two investigation years was represented by six independent samples with a total weight of 120 g. The purchased herb samples were milled to a powder in the laboratory mill GM-200 (Retsch GmbH, Berlin, Germany) and stored at −80 °C to avoid losing bioactive compounds before analyses.

### 2.1. Polyphenols Analysis

Polyphenols were measured by the High-Performance Liquid Chromatography (HPLC) method [17]. Powdered herb samples (100 mg) were extracted with 80% of methanol in an ultrasonic bath (30 °C, 10 min, 5.5 kHz). Next, samples were centrifuged (2 °C, 10 min, 6000 rpm). The supernatant was then collected and re-centrifuged (0 °C, 5 min, rpm). One milliliter of supernatant was transferred to HPLC-vials and used for analysis. For analysis purposes, an I-IPLC-set up was used, consisting of two LC-20AD pumps, a CMB-20A system controller, an SIL-20AC autosampler, an ultraviolet-visible SPD-20AV detector, an CTD-20AC oven, and a Phenomenex Fusion-RP 80A column (250 × 4.60 mm), all from Shimadzu (Shimpo, Warsaw, Poland). The gradient mobile phase contained 10% (Phase A) and 55% (Phase B) of acetonitrile. After the mixing of acetonitrile and water in appropriate proportions, orthophosphoric acid (85%) was added and the pH of the solution was measured simultaneously. After obtaining a stable value (3.0), the phases were ready to flow: 1 mL min<sup>−1</sup> and time program of 1.00–22.99 min, Phase A 95% and Phase B 5%; 23.00–27.99 min Phase A 50% and Phase B 50%; 28.00–28.99 min Phase A 80% and Phase B 20%; and 29.00–38.00 min Phase A 95 % and Phase B 5%. The wavelengths used for detection were 250 nm for flavonoids (quercetin-3-O-rutinoside, kaempferol-3-O-glucoside, myricetin, quercetin, quercetin-3-O-glucoside, apigenin, and kaempferol) and 370 nm for phenolic acids (gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic). The phenolic compounds (quercetin-3-O-rutinoside, kaempferol-3-O-glucoside, myricetin, quercetin, quercetin-3-O-glucoside, apigenin, kaempferol, gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic) were identified based on Fluka and Sigma Aldrich (Warsaw, Poland) external standards with a purity of 99.5%.

### 2.2. Carotenoids Analysis

Carotenoids and chlorophylls were measured by the HPLC method [17]. The examined powdered herb samples (100 mg) were extracted with cold acetone, and then magnesium carbonate was added. The samples were incubated in a cold ultrasonic bath (0 °C, 15 min). After extraction, the samples were centrifuged (5500 rpm, 2 °C, 10 min). From the test tube, 1 mL of supernatant was collected and re-centrifuged (rpm, 3 °C, 5 min). The gradient mobile phase contained 90% acetonitrile and 10%

methanol (Phase A) and 68% methanol and 32% ethyl acetate (Phase B), with flow of 1 mL min<sup>-1</sup> and time program of 1.00–14.99 min Phase A 100%; 15.00–22.99 min Phase A 40% and Phase B 60%; and 24.00–28.00 min Phase A 100%. The wavelengths used for detection were 445 nm (for lutein and zeaxanthin) and 450 nm (for beta-carotene and chlorophylls a and b). The carotenoids and chlorophylls were identified based on Fluka and Sigma Aldrich external standards with a purity of 99.5% (Poland).

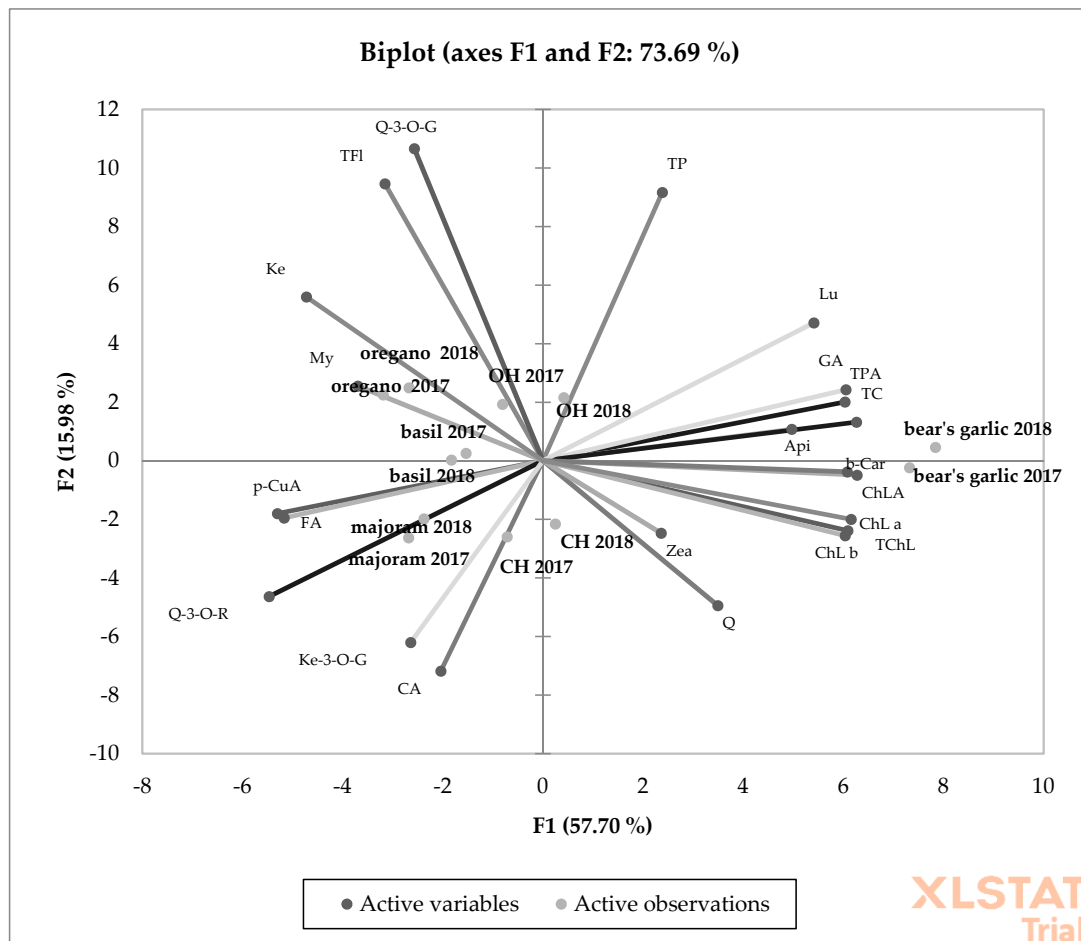
### 2.3. Statistical Analysis

The obtained results were statistically analyzed. Two-way analysis of variance with the use of Tukey's test ( $p = 0.05$ ) using Statgraphics® Centurion 15.2.11.0 software (StatPoint Technologies, Inc., Warrenton, VA, USA). Factors within the experiment were the method of production (organic or conventional) and the kind of herbs (basil, bear's garlic, marjoram, and oregano). The number of samples per system of production was  $n = 24$  and the number of samples per herb was  $n = 12$  in each year of the study. The lack of statistically significant differences ( $p > 0.05$ ) is described in tables as N.S. Different letters within a row indicate statistically significant differences on the level ( $p < 0.05$ ). All interaction between species and production systems are presented in Supplementary material (Tables S1–S3). PCA (Principal Component Analysis) was performed with XLSTAT Software package (XLSTAT, 2020, New York, NY, USA).

## 3. Results

In both years of experiment, organic herbs contained significantly more total polyphenols (1769.9 and 1821.2 mg 100 g<sup>-1</sup>, respectively) compared to conventionally grown herbs (1175.0 and 1243.1 mg 100 g<sup>-1</sup>, respectively). In the organic samples, we observed significantly more total phenolic acids and total flavonoids compared to conventional herbs. In both years of the experiment, the highest total polyphenols concentration was observed in oregano (2006.4 and 2007.6 mg 100 g<sup>-1</sup>, respectively) (Tables 1 and 2). In both years of the experiment, bear's garlic was characterized by the higher level of total phenolic acids (1291.9 and 1482.9 mg 100 g<sup>-1</sup>, respectively). In both years of the experiment, bear's garlic contained significantly more gallic (1157.9 and 1328.3 mg 100 g<sup>-1</sup> respectively) and chlorogenic acids (129.4 and 150.1 mg 100 g<sup>-1</sup> g, respectively). In the conventional herbs, we observed significantly more chlorogenic (42.0 and 64.6 mg 100 g<sup>-1</sup>) and caffeic (7.0 and 6.9 mg 100 g<sup>-1</sup>) acids compared with organic herbs in both experimental years (Tables 1 and 2). The system of production did not influence the *p*-coumaric and ferulic acid concentration in both years of the experiment. The levels of ferulic (18.2 mg 100 g<sup>-1</sup>) and *p*-coumaric (55.4 mg 100 g<sup>-1</sup>) in 2017 and *p*-coumaric (45.3 mg 100 g<sup>-1</sup>) and ferulic (14.8 mg 100 g<sup>-1</sup>) acids in 2018 were significantly highest in marjoram compared with the other herbs. The total flavonoid concentration was the highest in oregano (1410.5 and 1343.6 mg 100 g<sup>-1</sup>, respectively) compared with the other herbs. In the case of individual flavonoid concentrations, quercetin (25.4 and 29.5 mg 100 g<sup>-1</sup> in 2017 and 2018, respectively) was noted in significantly greater amount in conventional herbs than in organic herbs, as were quercetin-3-*O*-rutinoside (17.6 and 14.1 mg 100 g<sup>-1</sup>, respectively) and kaempferol-3-*O*-glucoside (321.0 and 336.5 mg 100 g<sup>-1</sup>, respectively) (Tables 1 and 2). Quercetin-3-*O*-glucoside (884.5 and 821.6 mg 100 g<sup>-1</sup>, respectively) and kaempferol (31.9 and 28.9 mg 100 g<sup>-1</sup>, respectively) concentrations were significantly higher in organic herbs than in conventional herbs in both years of the experiment (Tables 1 and 2). Total carotenoids (27.8 and 28.9 mg 100 g<sup>-1</sup>, respectively), but not beta-carotene (17.7 and 18.2 mg 100 g<sup>-1</sup>, respectively) concentration, was significantly higher in conventional herbs than in organic herbs in both experimental years, but xanthophylls, such as lutein (11.7 and 12.9 mg 100 g<sup>-1</sup>, respectively) and zeaxanthin (1.8 and 1.8 mg 100 g<sup>-1</sup>, respectively), were more abundant in organic herbs compared to conventional ones. We observed that all carotenoids (except of zeaxanthin in 2017 in marjoram) occurred in significantly greater amount in bear's garlic compared with the other herbs. In the case of the total chlorophyll colorant, as well as the individual phases (chlorophyll a and b), the levels were significantly higher in conventional herbs than in organic herbs in both years of the experiment (Tables 1 and 2). PCA showed a high and significant overall variation in both year of experiment. Overall, 73.69% was explained

by PC1 and PC2 (Figure 1). The degree of dependence between the conventional herbs and the factors marked as total polyphenol (TP), total phenolic acid (TPA), quercetin (Q), gallic acid (Gal), apigenin (Api), luteolin (Lu) and all chlorophyll compounds contents were particularly important, in both experimental years. It is worth pointing out that organic herbs were located in different sections of the graph, and were mostly dependent on total polyphenols (TP), total phenolic acids (TPA), and quercetin-3-*O*-glucoside (Q-3-*O*-G).



**Figure 1.** PCA analysis showing the relationship between the chemical composition and herbs origin (organic and conventional) as well species (oregano, basil, marjoram, and bear's garlic) in two experimental years total polyphenols (TP), total phenolic acids (TPA), gallic acid (GA), chlorogenic acid (ChLA), caffeic acid (CA), *p*-coumaric acid (p-CuA), ferulic acid (FA), total flavonoids (TFL), quercetin-3-*O*-rutinoside (Q-3-*O*-R), kaempferol-3-*O*-glucoside (Ke-3-*O*-G), myricetin (My), quercetin (Q), quercetin-3-*O*-glucoside (Q-3-*O*-G), apigenin (Api), kaempferol (Ke), total carotenoids (TC), lutein (Lu), zeaxanthin (Zea), beta-carotene (b-Car), total chlorophylls (TChL), chlorophyll b (ChL b), and chlorophyll a (ChL a).

**Table 1.** The content of bioactive compounds (in mg 100 g<sup>−1</sup> of product) in selected spices from organic and conventional production in 2017.

Bioactive Compounds/Experimental Combination	Organic Herbs	Conventional Herbs	Basil	Bears' Garlic	Marjoram	Oregano	<i>p</i> -Value	
							System	Species
total polyphenols	1769.9 ± 160.2 A	1175.0 ± 78.4 B	1001.3 ± 80.3 d	1772.7 ± 18.6 b	1109.4 ± 35.0 c	2006.4 ± 296.5 a	<0.0001	<0.0001
total phenolic acids	735.5 ± 76.1 A	631.1 ± 74.9 B	358.2 ± 15.0 c	1291.7 ± 22.8 a	487.4 ± 11.1 c	595.9 ± 46.3 b	0.0003	<0.0001
gallic acid	663.4 ± 72.6 A	543.0 ± 65.6 B	329.8 ± 14.2 c	1158.0 ± 28.4 a	403.4 ± 12.6 c	521.5 ± 50.8 b	0.0001	<0.0001
chlorogenic acid	28.8 ± 8.6 B	42.0 ± 13.6 A	2.4 ± 0.5 c	129.4 ± 8.1 a	1.3 ± 0.0 c	8.3 ± 0.2 b	<0.0001	<0.0001
caffeic acid	3.9 ± 0.5 B	7.0 ± 0.7 A	2.5 ± 0.1 c	4.3 ± 0.5 b	9.1 ± 0.2 a	6.1 ± 1.2 b	<0.0001	<0.0001
<i>p</i> -coumaric acid	29.8 ± 4.6 A	29.4 ± 4.7 A	17.8 ± 1.0 b	N.D.	55.4 ± 2.0 a	45.2 ± 2.7 a	N.S.	<0.0001
ferulic acid	13.0 ± 1.2 A	12.8 ± 1.2 A	5.6 ± 0.3 b	N.D.	18.2 ± 0.7 a	14.8 ± 0.9 a	N.S.	<0.0001
total flavonoids	1034.4 ± 150.1 A	543.9 ± 25.6 B	643.1 ± 70.0 b	481.0 ± 7.9 c	621.9 ± 27.4 b	1410.5 ± 250.7 a	<0.0001	<0.0001
quercetin-3- <i>O</i> -rutinoside	9.5 ± 0.2 B	17.6 ± 0.8 A	10.6 ± 0.5 b	N.D.	15.9 ± 1.6 a	14.1 ± 1.5 b	<0.0001	<0.0001
kaempferol-3- <i>O</i> -glucoside	59.5 ± 13.7 B	321.0 ± 28.2 A	6.4 ± 0.5 b	N.D.	166.5 ± 44.6 a	160.2 ± 23.0 a	<0.0001	<0.0001
myricetin	82.1 ± 6.9 A	55.3 ± 0.9 B	N.D.	N.D.	61.4 ± 15.8 b	76.0 ± 8.0 a	<0.0001	<0.0001
quercetin	15.6 ± 2.1 B	25.4 ± 2.4 A	11.0 ± 2.6 c	27.8 ± 1.4 a	23.1 ± 4. b	20.0 ± 2.4 c	0.003	0.0045
quercetin-3- <i>O</i> -glucoside	884.5 ± 137.2 A	369.3 ± 13. B	592.3 ± 70.5 b	392.1 ± 11.1 c	340.3 ± 6.0 c	1182.9 ± 236.2 a	<0.0001	<0.0001
apigenin	17.5 ± 6.2 A	13.1 ± 4.6 B	N.D.	61.1 ± 2.6	N.D.	N.D.	<0.0001	<0.0001
kaempferol	31.9 ± 1.9 A	18.4 ± 2.3 B	24.4 ± 0.3 b	N.D.	14.7 ± 3.6 c	36.2 ± 2.4 a	<0.0001	<0.0001
total carotenoids	28.4 ± 1.2 A	27.8 ± 1.6 B	23.5 ± 0.2 c	39.4 ± 1.0 a	24.0 ± 0.3 b	25.5 ± 0.7 b	0.0063	<0.0001
lutein	11.7 ± 0.7 A	8.5 ± 0.1 B	8.9 ± 0.2 b	13.3 ± 1.3 a	9.0 ± 0.2 b	9.2 ± 0.5 b	<0.0001	<0.0001
zeaxanthin	1.8 ± 0.03 A	1.6 ± 0.04 B	1.7 ± 0.02 a	1.8 ± 0.04 a	1.8 ± 0.03 a	1.4 ± 0.08 b	0.001	0.0013
beta-carotene	15.0 ± 0.5 B	17.7 ± 1.5 A	12.9 ± 0.1 c	24.3 ± 1.9 a	13.2 ± 0.1 b	14.9 ± 0.3 b	0.0022	<0.0001
total chlorophylls	120.0 ± 11.4 B	184.1 ± 38.3 A	70.6 ± 4.3 c	354.9 ± 46.5 a	106.1 ± 4.9 b	76.5 ± 7.4 c	0.0005	<0.0001
chlorophyll b	83.4 ± 8.5 B	128.3 ± 26.5 A	50.4 ± 3.9 c	247.6 ± 30.9 a	74.6 ± 3.4 b	50.8 ± 4.8 c	0.0005	<0.0001
chlorophyll a	36.6 ± 3.1 B	55.7 ± 12.3 A	20.2 ± 0.5 c	107.3 ± 15.7 a	31.5 ± 1.7 b	25.7 ± 2.6 c	0.0022	<0.0001

Data are presented as the mean ± SE with ANOVA *p*-Value. Means in rows followed by the same letter are not significantly different at the 5% level of probability (*p* < 0.05). N.S., not significant; n, number of samples (replications), n = 12 (species), n = 24 (system); N.D., not detected (below LOD/LOQ).

**Table 2.** The content of bioactive compounds (in mg 100 g<sup>−1</sup> of product) in selected spices from organic and conventional production in 2018.

Bioactive Compounds/Experimental Combination	ORG	CONV	Basil	Bears' Garlic	Marjoram	Oregano	<i>p</i> -Value System
total polyphenols	1821.2 ± 158.5 A	1243.1 ± 91.5 b	1008.8 ± 16.7 c	1968.2 ± 12.1 a	1145.0 ± 12.8 b	2006.5 ± 18.9 a	<0.0001
total phenolic acids	830.4 ± 88.9 A	710.2 ± 87.4 b	402.4 ± 18.4 d	1482.7 ± 15.2 a	533.3 ± 12.5 d	662.9 ± 12.2 c	0.0003
gallic acid	760.9 ± 83.3 A	622.9 ± 75.3 b	378.3 ± 18.3 d	1328.3 ± 151 a	462.8 ± 12.8 c	598.2 ± 11.9 b	0.0001
chlorogenic acid	33.3 ± 10.0 B	64.6 ± 17.1 A	4.6 ± 0.2 c	150.1 ± 0.2 a	1.4 ± 0.1 d	9.6 ± 0.1 b	<0.0001
caffeic acid	3.9 ± 0.5 B	6.9 ± 0.7 A	2.4 ± 0.1 d	4.2 ± 0.1 c	8.9 ± 0.1 a	6.0 ± 0.1 b	<0.0001
<i>p</i> -coumaric acid	24.4 ± 3.8 A	24.1 ± 3.8 A	14.7 ± 0.1 c	N.D.	45.3 ± 0.3 a	37.0 ± 1.0 b	N.S.
ferulic acid	10.6 ± 0.9 A	10.4 ± 1.0 A	4.6 ± 0.1 c	N.D.	14.8 ± 0.1 a	12.1 ± 0.3 b	N.S.
total flavonoids	990.8 ± 140.6 A	532.9 ± 26.0 B	606.4 ± 2.5 b	485.5 ± 3.3 c	611.7 ± 0.3 b	1343.6 ± 6.7 a	<0.0001
quercetin-3- <i>O</i> -rutinoside	7.6 ± 0.1 B	14.1 ± 0.7 A	8.5 ± 0.1 c	N.D.	12.8 ± 0.2 a	11.3 ± 0.1 b	<0.0001
kaempferol-3- <i>O</i> -glucoside	62.4 ± 15.3 B	336.5 ± 0.8 A	6.7 ± 0.3 b	N.D.	174.5 ± 0.1 a	168.0 ± 4.3 a	<0.0001
myricetin	61.6 ± 9.1 A	39.3 ± 9.9 B	N.D.	8.5 ± 0.2 b	63.8 ± 0.7 a	79.0 ± 0.1 a	<0.0001
quercetin	18.1 ± 2.5 B	29.5 ± 2.8 A	12.7 ± 0.1 c	32.4 ± 0.4 a	26.8 ± 0.1 b	23.2 ± 0.1 b	0.003
quercetin-3- <i>O</i> -glucoside	821.6 ± 127.5 A	342.9 ± 13.0 B	550.1 ± 2.6 b	364.2 ± 3.0 c	316.0 ± 0.5 c	1098.8 ± 5.2 a	<0.0001
apigenin	19.8 ± 7.0 A	14.8 ± 5.2 B	N.D.	69.2 ± 0.2	N.D.	N.D.	<0.0001
kaempferol	32.6 ± 3.8 A	28.6 ± 1.7 B	31.8 ± 0.3 b	11.3 ± 0.1 c	35.5 ± 0.1 b	47.3 ± 0.3 a	<0.0001
total carotenoids	29.9 ± 1.4 A	29.0 ± 1.7 B	24.3 ± 0.2 b	42.1 ± 0.1 a	24.8 ± 0.3 b	26.6 ± 0.6 b	0.0063
lutein	12.9 ± 0.9 A	9.2 ± 0.2 B	9.5 ± 0.1 b	14.9 ± 0.1 a	9.8 ± 0.1 b	9.9 ± 0.2 b	<0.0001
zeaxanthin	1.8 ± 0.01 A	1.6 ± 0.01 B	1.7 ± 0.01 a	1.7 ± 0.01 a	1.8 ± 0.02 a	1.4 ± 0.1 b	0.0009
beta-carotene	15.3 ± 0.5 B	18.2 ± 1.7 A	13.0 ± 0.1 c	25.5 ± 0.1 a	13.3 ± 0.1 c	15.2 ± 0.3 b	0.0019
total chlorophylls	107.6 ± 10.0 B	164.2 ± 34.4 A	63.7 ± 1.8 c	315.2 ± 4.4 a	95.2 ± 2.5 b	69.5 ± 5.5 c	0.0005
chlorophyll b	71.3 ± 7.1 B	108.8 ± 22.2 A	43.6 ± 1.6 c	208.7 ± 1.1 a	63.9 ± 2.2 b	44.0 ± 3.6 c	0.0005
chlorophyll a	36.4 ± 3.1 B	55.3 ± 12.2 A	20.1 ± 0.5 c	106.5 ± 3.4 a	31.3 ± 0.5 b	25.5 ± 1.9 c	0.0022

Data are presented as the mean ± SE with ANOVA *p*-Value. Means in rows followed by the same letter are not significantly different at the 5% level of probability (*p* < 0.05). N.S., not significant; n, number of samples (replications), n = 12 (species), n = 24 (system); N.D., not detected (below LOD/LOQ).

#### 4. Discussion

Herbs are a good source of phenolic acids in our diet. The obtained results show that organic herbs contained significantly more total phenolic acids than conventional herbs. This finding is similar to what was reported in some experiments which showed the mean value of total phenolic acids in fresh medicinal plants (organic) was 33.49 mg 100 g<sup>-1</sup> FW and (conventional) 32.52 mg 100 g<sup>-1</sup> FW, as well as in herbs (organic) 31.10 mg 100 g<sup>-1</sup> FW and (conventional) 26.50 mg 100 g<sup>-1</sup> FW [18,19]. Unfortunately, many research results presented in the literature regarding the impact of organic and conventional cultivation on the content of phenolic acids in herbs do not explain the mechanisms of this phenomenon [18,19]. Organic herbs are cultivated under strict rules when easily available fertilizers cannot be used. We can only suppose that higher level of phenolic acids in our organic herbs is an effect of different kinds of fertilization, especially potassium fertilization. In another presented experiment, organic potato was characterized by higher phenolic acids concentration and the authors explained this phenomenon by different fertilization regime [20]. In one experiment with organically and conventionally grown leafy vegetables, the higher amount of phenolic acids in organic plants was explained as a plant reaction for a biotic and abiotic stresses [10]. The species and variety of plant significantly influence phenolic acid accumulation in herbs. As we demonstrated in the experiment, two examined herbs (marjoram and bear's garlic) were characterized by the highest level of identified phenolic acids, while basil and oregano contained significantly lower individual phenolic acids. The diversification of the concentration of phenolic acids between species is an effect of genetic factors. Different polyphenol compounds such as phenolic acids play important roles in plant-microbe interactions/symbiosis. Phenolic acids act as signaling molecules in the initiation of plant-microbe symbioses [21]. In organic systems, plants are cultivated with organic fertilizers and the biological activity of the soil is greater than in conventional farming. The higher level of phenolic acids in organic plants is an effect of "chemical signaling" between plant and soil microbes [22]. We found the higher level of total phenolic acids and gallic acid in organically-produced herbs in both years of the experiment. Gallic acid is a very important phenolic acid induced by Rhizobia in rice resistant to Rhizoctonia [23]. The higher concentration of ferulic acid could stimulate root growth inhibition and lead to slower plant growth and plant dry mass accumulation [24]. This observation is in accordance with the present results. It is worth noting that conventional herbs with a higher ferulic acid concentration also had a lower concentration of gallic acid. Flavonoids have antioxidant abilities and protect plants from various biotic and abiotic stresses. The role of secondary metabolic pathways in plant responses is to cope with oxidative stress, which is responsible for the synthesis of flavonoids [25].

Organic herbs and medicinal plants contain a higher level of flavonoids [18,19]. The higher concentration of flavonoids in fresh organic herbs and medicinal plants is an effect of agricultural condition used in that system. Organic basil contained 7.03 mg g<sup>-1</sup> FW flavonoids in control combination, 8.23 mg g<sup>-1</sup> in 100% compost, and 11.48 mg g<sup>-1</sup> FW in compost with bioactivator [26]. The authors found a link between organic matter applying and the concentration of flavonoids in basil. They may be attributed to the C/N theory. In natural conditions, when nitrogen is readily available (conventional agriculture), plants would primarily make compounds with high nitrogen concentration, (proteins, amino acids for growth, and N-containing secondary metabolites such as alkaloids). When nitrogen availability is limiting for growth (organic agriculture), metabolism changes more towards carbon (C) containing compounds (flavonoids) [17]. In the presented experiment, organic herbs contained significantly more flavonoids compared to conventional herbs in both years of the experiment. The organic herbs were characterized by a higher level of quercetin-3-O-glucoside, quercetin-3-O-rutinoside and kaempferol-3-O-glucoside and a lower level of aglycone quercetin compared to conventional herbs. The oxidation rates of individual flavonoids in plant tissue were in the order: quercetin > kaempferol > quercetin glycoside > kaempferol glycoside [27]. The different ratio of glycone and aglycone form of flavonoids is connected with their oxidation rate. In plants cultivated in organic condition, the oxidation process is more effective [28]. The differences in the

flavonoid concentration between herbs species is the result of genetic differences. Particular species have different capacities for the production and accumulation of phenolic compounds. Within one herb family, there may be significant differences in bioactive compound concentration [29]. Three of four herbs studied here (basil, marjoram, and oregano) belong to the same family (Lamiaceae), but the variation in total flavonoids concentration was very high. Oregano contained 1410.5 mg 100 g<sup>-1</sup> in 2017 and 1344.8 mg 100 g<sup>-1</sup> in 2018, while marjoram contained 621.9 mg 100 g<sup>-1</sup> in 2017 and 611.7 mg 100 g<sup>-1</sup> in 2018. Similar results are presented in another experiment [19], where the authors analyzed the concentration of total and individual flavonoids in sage, lemon balm, and peppermint. Those species also belong to the Lamiaceae family. The highest number of total flavonoids found was in sage (153.74 mg 100 g<sup>-1</sup> DW), followed by peppermint (113.82 mg 100 g<sup>-1</sup> DW), and the least in lemon balm (105.87 mg 100 g<sup>-1</sup> DW). It is worth noting that the oregano was obtained from a biodynamic farming system, where only organic fertilization is allowed [30]. In another experiment with leafy vegetables (chicory), biodynamic plants produced much more flavonoids than conventional ones [31].

In the present study, the examined herbs consisted in majority of plant leaves, which contain a large amount of the green pigment, chlorophyll. It is well known that there is a link between chlorophyll concentration and the level of nitrogen fertilization [32]. Organic plants are cultivated only with organic fertilizers and with lower level of nitrogen fertilization compared to conventional herbs. White cabbage cultivated in organic farming contained significantly less total chlorophyll and chlorophyll fractions (a and b) compared to that cultivated by conventional methods [17]. In another experiment, a link was found between a higher level of nitrogen fertilization and chlorophyll concentration in maize leaves [33]. The higher variation between the examined herbs in the case of total chlorophyll concentration is an effect of plant genotype. The situation is similar as with total flavonoids concentration. Despite belonging to the same botanical family, the herbs contained different amounts of chlorophyll (Tables 1 and 2). In the presented experiment, the highest levels of total chlorophyll and its fractions (a and b) were found in bear's garlic leaves in both years. In another experiment, 479.72 mg 100 g<sup>-1</sup> DW of total chlorophyll including chlorophyll a (374.95 mg 100 g<sup>-1</sup>) and chlorophyll b (104.77 mg 100 g<sup>-1</sup>) were found [34]. In foliar plants, green chlorophyll is strictly connected with beta-carotene. Carotenoids are synthesized via the general biosynthetic pathway within the chloroplasts of plants. The effect of UV-B radiation increased carotenoid accumulation and total antioxidant capacity in tobacco (*Nicotiana tabacum* L.) leaves [35].

Higher levels of active photosynthetic pigments were positively correlated with beta-carotene concentration. Because we found a high level of chlorophyll in conventional herbs, the level of beta-carotene was also higher in conventional plants (Tables 1 and 2). Organic medicinal plants contained significantly lower total carotenoids and beta-carotene compared to conventional plants [18]. Lutein is one of the most abundant carotenoids found in plant leaf tissue [36]. The higher concentration of lutein in leafy plant tissue is an effect of UV-radiation because lutein, as well as zeaxanthin, protects chloroplasts against photooxidation [37]. We found a higher concentration of xanthophylls (lutein and zeaxanthin) in organic herbs in both years of the experiment compared to conventional herbs. This finding can be explained by using an organic fertilization, which stimulates lutein concentration in the leafy plants. Organic lettuce was characterized by a higher lutein concentration (12.7 µg g<sup>-1</sup> FW) compared to the conventional one (6.24 µg g<sup>-1</sup> FW) [38]. The use of organic fertilization in lettuce cultivation increases the concentration of lutein in the leaves [39].

## 5. Conclusions

Our study demonstrated that organic herbs in comparison to conventional ones were characterized by a significantly higher level of most of the identified polyphenols and carotenoids. On the other hand, conventional herbs contained more quercetin, kaempferol-3-O-glucoside, chlorophylls, and total carotenoids. The results obtained here are extremely valuable because of their usefulness to the consumer, who is the ultimate user of organic herbs.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3417/10/10/3468/s1>: Table S1: The levels of LOD (limit of detection) and LOQ (limit of quantification) for all compounds used in experiment; Table S2. The concentration of individual bioactive compounds (in mg 100 g<sup>-1</sup> of product ) in selected herbs from organic and conventional production in 2017, mean values  $\pm$  standard error; Table S3. The concentration of individual bioactive compounds (in mg 100 g<sup>-1</sup> of product ) in selected herbs from organic and conventional production in 2018, mean values  $\pm$  standard error.

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