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# Antioxidative and antibacterial properties of organically grown thyme (*Thymus* sp.) and basil (*Ocimum basilicum* L.)

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**Abstract:** The biomass per plant and total phenolics (TPH) of ethanol extracts of 3 species of thyme, *Thymus vulgaris*, *T. serpyllum*, and *T. citriodorus,* and 3 varieties of basil *(Ocimum basilicum*), Genovese, Thai, and Cinnamon, were investigated. All were grown organically. The 2 herbs that showed the greatest biomass were *T. citriodorus (*26.5 g/plant*)* and *O. basilicum* var. Cinnamon (105 g/plant). For these, total flavonoids (TFL), total flavones and flavonols (TFF), and total flavanones and dihydroflavonols (TFDH) were also determined for their ethanol extracts. Furthermore, the antioxidant potential (AOP) and antibacterial activity were determined against foodborne bacteria: *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and verotoxin-producing *Escherichia coli* (VTEC). The highest TPH (36 mg chlorogenic acid equivalents per gram of fresh weight) was seen for *T. citriodorus* collected in 2010. The thyme ethanol extracts contained more TPH and TFL, and their subgroups of TFF and TFDH, than the ethanol extracts from basil, and had AOP comparable to the ethanol extracts from basil, although all of them were lower than the synthetic antioxidant, t-butylated hydroxytoluene. Drying of these herbs decreased TFF and TFDH, while TPH and TFL remained unchanged (for *T. citriodorus*) or even increased (for Cinnamon basil), and AOP was higher than that of the ethanol extracts from the frozen herbs. The antimicrobial activities of these ethanol extracts depended mainly on the bacterial target. They were weak against gram-negative VTEC, while their effects against *C. jejuni*, *S. aureus*, and *L. monocytogenes* correlated with their TPH and chemical compositions.

**Key words:** Antimicrobials, antioxidant potential, basil, organic grow, phenolics, thyme

## 1. Introduction

The genus *Thymus* (Lamiaceae, or Labiatae) includes about 350 species of aromatic plants that are grown in Europe, North America, and Asia. Three species were included in this study: *Thymus vulgaris*, *Thymus serpyllum*, and *Thymus citriodorus*. *T. vulgaris*, or common thyme, is a low-growing, herbaceous, evergreen, perennial shrub. *T. serpyllum*, or wild thyme, is grown in the same area but at higher altitudes (Nikolić et al., 2014). *T. citriodorus*, or lemon thyme, is a hybrid between *T. pulegioides* and *T. vulgaris*. *T. citriodorus* smells and tastes like lemon. Leaves of all 3 species are used in cooking and their phenolics act as antimicrobials and stimulants (Giordani et al., 2004; Mata et al., 2007).

The genus *Ocimum* (Lamiaceae) consists of at least 150 species and numerous varieties (Labra et al., 2004) that were originally grown in Asia, Africa, and Central and South America. Three varieties of basil (*Ocimum basilicum*) were used in the present study: Genovese basil, Thai basil, and Cinnamon basil. Genovese basil is grown in regions with mild temperatures. Thai basil's flavor is more stable during cooking than that of Cinnamon basil (Simon et al., 1999), which is used in traditional medicine in addition to cooking (Fratianni et al., 2017).

The preserving effects of many plant spices and herbs are mainly due to the diverse phenolics they contain, which can have antioxidative and antimicrobial properties. The family Lamiaceae has long been recognized as a rich source of phenolic acids, phenolic monoterpenes, and flavonoids (Phippen and Simon, 1998; Kwee and Niemeyer, 2011; Park, 2011; Jabri-Karoui et al., 2012). The growth, biomass, and phenolic content can be markedly affected by the geographical environment, the local environment where the plant is grown, physical and chemical characteristics

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of the soil, seed source, plant age, parts of the plant that are used, and the isolation method used (Sharafzadeh et al., 2011; Flanigan and Niemeyer, 2014).

Air drying is a traditional postharvest method to protect against deterioration and spoilage and to prolong the shelf life of herbs. However, the drying process itself can affect the total phenolics (TPH), antioxidant potential (AOP), and antimicrobial activities (Mansour, 2016; Parmar et al., 2017).

The aim of the present study on these 3 species of *Thymus* and these 3 varieties of *O. basilicum* was to investigate the biomass per plant and to determine the TPH content. These plants were all grown under organic conditions in the same location and under the same soil conditions. Furthermore, for the herbs with the greatest biomass, which were lemon thyme and Cinnamon basil, the total flavonoids (TFL), total flavones and flavonols (TFF), and total flavanones and dihydroflavonols (TFDH) were also determined, as well as the AOP and the antibacterial activities of the ethanol extracts against different foodborne pathogenic bacteria, which included *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and verotoxin-producing *Escherichia coli* (VTEC). To determine how air drying affects the TPH composition and biological properties of these ethanol extracts, both frozen and dried herbs were analyzed. The relationships between biomass, TPH content, flavonoids composition, AOP, and antimicrobial properties were then examined.

## 2. Materials and methods

### 2.1. Chemicals

2,4-Dinitrophenylhydrazine, chlorogenic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), naringenin, resazurin, quercetin, and t-butylhydroxytoluene (BHT) were from Sigma Aldrich (Germany). Rutin was from Merck (Germany), and Folin–Ciocalteu reagent was from Fluka (Switzerland). The other chemicals and solvents were of analytical purity.

### 2.2. Plant materials

Three species of thyme (*Thymus*) and 3 varieties of basil (*O. basilicum*) were collected in 2010, and then lemon thyme (*T. citriodorus*) and Cinnamon basil (*O. basilicum* 'Cinnamon') were collected in 2013 (Table 1). The fresh thyme contained 70% moisture and the fresh basil contained 80% moisture.

### 2.3. Growing the plant material

Seedlings were grown according to the guidelines for organic production (thyme from cuttings; basil from seeds). The sowing/planting in trays was carried out in September 2009 and 2012, with transplanting to pots early the following spring (Table 1). The seedlings were transplanted to the experimental field of the Slovenian Institute of Hop Research and Brewing at the beginning of May 2010 and 2013.

The size of the plot for all of the herbs included was 1.5 m × 2 m, the within-row distance was 15 cm, and the distance between the rows was 30 cm. The plants were grown according to organic farming guidelines. No diseases or pests were detected during the growth seasons. Manual hoeing was performed at the beginning of June. No fertilizing was performed. The experimental plants were irrigated because of the extremely dry conditions at the end of April 2010; in 2013, no irrigation was necessary. The harvest for the aboveground biomass in the first experiment was completed on 1 July 2010, with the harvest in the second experiment completed on 19 July 2013, which was just before bud formation in both cases.

### 2.4. Growth conditions

The substrate used in the trays and pots was S25 – Biotray + Ecpo-mix 70L/45EP – Gramoflor (Germany). The soil in the experimental field was well supplied in terms of phosphate ($P_2O_5$, 570 mg/kg soil), potassium ($K_2O$, 271 mg/kg soil; determined according to the Al analytical method for soil analysis of Hodnik, 1988), and organic matter (32 mg/kg soil; determined according to analytical method ISO 14235). The soil pH (6.7, in KCl; determined according to analytical method ISO 10390) was suitable for growing herbs, so no liming was carried out. All of the herbs investigated during both of the years (i.e. 2010, 2013) were grown in the same field.

The weather conditions in the growing seasons of 2010 and 2013 are illustrated in Figure 1. In both seasons there were less rainfall and higher temperatures compared to the

**Table 1.** Growth of the herbs included in the present study.

| Herb | Common name | Cultivar/variety | Sown | Transplanted |
|---|---|---|---|---|
| Thyme | Common | *Thymus vulgaris* | September 2009 | February 2010 |
| | Wild | *Thymus serpyllum* | September 2009 | February 2010 |
| | Lemon | *Thymus citriodorus* | September 2009/2012 | February 2010/2013 |
| Basil | Genovese | *Ocimum basilicum* | February 2010 | End March 2010 |
| | Thai | *Ocimum basilicum* | February 2010 | End March 2010 |
| | Cinnamon | *Ocimum basilicum* | February 2010/2013 | End March 2010/2013 |

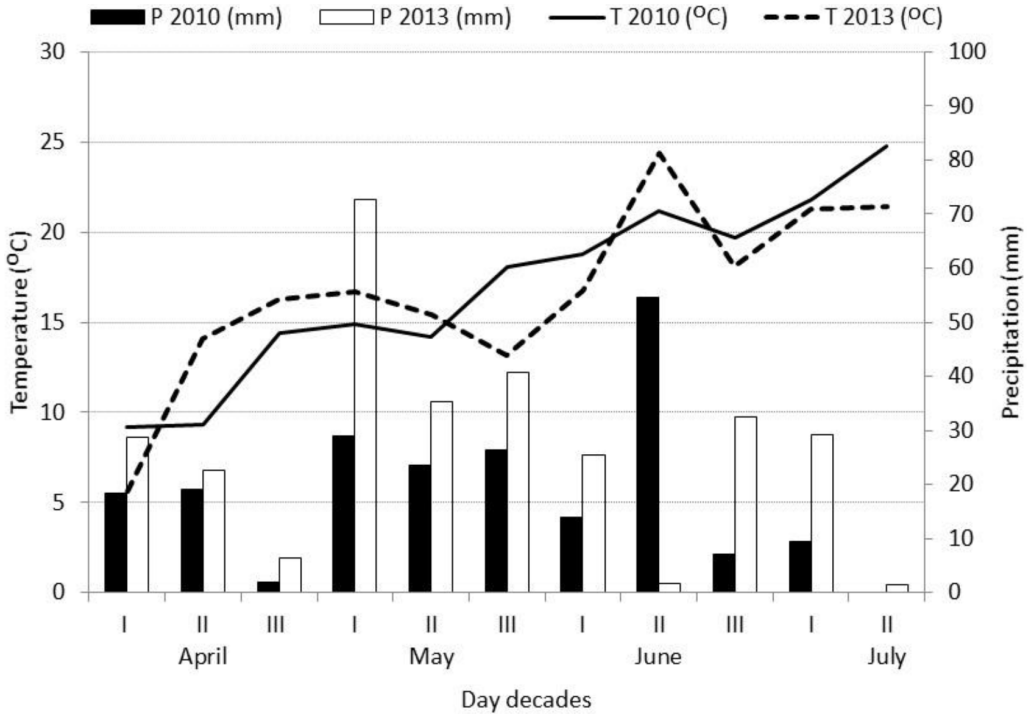


**Figure 1.** Temperature and precipitation of the experimental field (in Žalec, Slovenia) from April to July in 2010 and 2013 (I, II, III = day decades).

long-term mean. From 1 April 2010 to 1 July 2010, there was 194 mm of precipitation, compared to 297 mm of precipitation from 1 April 2013 to 19 July 2013. The mean daytime temperature was 15.5 °C during the 2010 growing season, versus 16.7 °C in 2013.

**2.5. Biomass determination and plant sample preparation**

The numbers of plants per plot were counted, and the cut fresh matter per plant was weighed for the biomass evaluation. Immediately after harvest, one half of the sample from each plot was frozen and stored in a freezer at -20 °C. The other half of the sample was dried at 40 °C for 2 days and then kept in a dry place in the dark.

**2.6. Extraction of phenolics**

For the ethanol extract preparation, the frozen herbs (3 g) were cut into small pieces and the dried herbs (1.5 g) were ground using a pestle and mortar. Then 30 mL of 96% ethanol was added to the samples, which were mixed at 60 °C overnight. The suspensions obtained were centrifuged at 3900 ´ *g* for 10 min (Centric 322B; Tehtnica, Slovenia). The supernatants were collected and used immediately for determination of the TPH. The rest of these samples were stored at -20 °C until further analysis.

**2.7. Total phenolics, total flavonoids, total flavones and flavonols, total flavanones and dihydroflavonols**

TPH was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965). TPH is expressed as chlorogenic acid equivalents (mg) per gram of fresh sample (mg CAE/g fw).

For the TFL determination, the method of Yang et al. (2004) was used. TFL is expressed as rutin equivalents (mg) per gram of fresh sample (mg RUE/g fw). TFF and TFDH were determined using the method of Popova et al. (2004). The TFF in the ethanol extracts is expressed as quercetin equivalents (mg) per gram of fresh sample (mg QUE/g fw). TFDH is expressed in naringenin equivalents (mg) per gram of fresh sample (mg NAE/g fw).

**2.8. Antioxidant potential**

The DPPH$^{\bullet}$ radical scavenging assay was used to evaluate the AOP of the ethanol extracts (Brand-Williams and Cuvelier, 1995). The AOP was expressed as TPH content in the reaction mixture necessary for a 50% reduction of the initial DPPH$^{\bullet}$ ($IC_{50}$; µg CAE/mL) and compared to that of BHT.

**2.9. Antibacterial activity**

For the testing of the antibacterial activities of the ethanol extracts of lemon thyme and Cinnamon basil, a panel of foodborne pathogenic bacteria was used. This comprised gram-positive *S. aureus* strains (5.1, 5.2, 5.3, 5.5, 5.6, ŽMJ 72) and *L. monocytogenes* strains (ŽM 52, ŽM 60, ŽM 62, ŽM 71, ŽM 72, L 95), and gram-negative *C. jejuni* strains (C2, C33, 11168, 375/05, 573/03, K49/4) and *E. coli* (VTEC) strains (2-3, 2-4, 62-4, 80-8, 80-21, 81-30) (Culture

Collection [ZIM], Biotechnical Faculty, University of Ljubljana, Slovenia). The growth media used were 'Agar Listeria according to Ottaviani and Agosti' (ALOA; Biolife, Italy), Baird Parker (Biolife, Italy), Karmali (Oxoid, UK), Mueller–Hinton agar (MHA; Oxoid, UK), and Mueller–Hinton broth (MHB, Oxoid, UK). The ethanol extracts of the phenolics for antimicrobial determination were vacuum dried at 35 °C, and the dry residues were dissolved in dimethyl sulphoxide. The minimum inhibitory concentrations (MICs) for the ethanol extracts were determined using the broth microdilution method (Klančnik et al., 2010). The MICs were the lowest concentrations where no viability was observed after 24 h on the basis of the metabolic activities, measured after addition of 10 µL of 0.252 mg resazurin/mL in MHB, with an incubation for 2 h in the dark (Klančnik et al., 2010). The MICs of the plant ethanol extracts were expressed as mg CAE/mL growth medium.

### 2.10. Statistical analysis

The biomass data were processed using Excel (Microsoft) and t-tests to define significant differences (P = 0.05) among the groups examined. All of the ethanol extracts were prepared in triplicate, and the determinations were performed in 3 repetitions. The reproducibility of ethanol extraction has been estimated to be within 8%. Data are reported as means ± standard deviation (SD). Pearson correlation coefficients (r) and coefficients of variation (CVs) were calculated using Excel (Microsoft). Data on TPH, TFL, TFF, and TFDH were processed by analysis of variance and differences between cultivars/varieties were detected by Duncan multiple range test (P = 0.05).

## 3. Results and discussion

Three species of thyme (common, wild, lemon thyme) and 3 varieties of basil (Genovese, Thai, Cinnamon basil) that were collected in 2010 were analyzed for biomass and TPH. Lemon thyme and Cinnamon basil showed the highest mean biomass among these investigated thyme species and basil varieties, respectively, and were collected again in 2013. This second set of samples was then used to determine TPH, TFL, TFF, TFDH, AOP, and antimicrobial activity.

### 3.1. Biomass

In 2010, the mean biomass of the aboveground plants of common thyme was 10.5 g/plant (range: 7.5-13.8 g/plant), of wild thyme 20.9 g/plant (range: 12.8-32.4 g/plant), and of lemon thyme 26.5 g/plant (range: 16.4-33.9 g/plant) (Table 2). There were significant differences in the biomass per plant between examined groups (t-tests; P = 0.05), except between wild thyme and lemon thyme.

The mean aboveground fresh biomass at the time of harvest of Genovese basil was 21.1 g/plant (range: 4.8-68.8 g/plant), of Thai basil 27.8 g/plant (range: 5.2-58.1 g/plant), and of Cinnamon basil 105.0 g/plant (range: 11.1-243.3 g/plant). There were no significant differences in the fresh biomass per plant between Genovese basil and Thai basil, while there was a significant difference in biomass between Genovese basil and Cinnamon basil and between Thai basil and Cinnamon basil.

**Table 2.** Biomass of fresh aboveground matter in 2010.

| Herb | Cultivar/variety | Fresh mass per plant (g) | |
|---|---|---|---|
| Thyme | Common | 10.5 | a* |
| | Wild | 20.9 | b |
| | Lemon | 26.5 | b |
| Basil | Genovese | 21.1 | a |
| | Thai | 27.8 | a |
| | Cinnamon | 105.0 | b |

*The same letter in the column within thyme and within basil plants means that there is no significant difference between cultivars/varieties (t-test, P = 0.05).

The variable yield per plant for Cinnamon basil and wild thyme was probably a consequence of the different stages that the young seedlings were in at the time of transplanting to the field: if the seedlings had inflorescences, these were cut off, and such seedlings usually develop more shoots and produce greater biomass, while the seedlings without inflorescences at the time of transplanting usually produce lower aboveground biomass before harvesting. There was also an impact of the relatively dry conditions during the growth season in 2010. The seedlings that took root in the ground better and more rapidly after transplanting had more opportunity to develop their root systems, which helped them to provide the plant with enough water for growth and development. This was probably also the reason for the variable biomass per plant for the varieties with higher aboveground biomass, such as Cinnamon basil.

### 3.2. Total phenolics, total flavonoids, total flavones and flavonols, and total flavanones and dihydroflavonols

In general, all varieties of basil had between 10% and 30% of the TPH contents of the thyme species. In 2010, TPH was significantly the highest in lemon thyme, followed by common and wild thyme, between which no significant difference was detected (Table 3). Among basil, Genovese basil had significantly the highest TPH, followed by Cinnamon basil and Thai basil.

In the ethanol extracts of the frozen Cinnamon basil from 2013, the TPH was comparable to that from 2010, while the ethanol extracts of frozen lemon thyme from 2013 had almost a third of the TPH content in the ethanol extracts from 2010. The lemon thyme plants were grown in

**Table 3.** Total phenolics of the ethanol extracts from the thyme and basil plants collected in 2010 and 2013.

| Herb | Cultivar/ | Sample | Total phenolics (mg CAE/g fw) | | | |
|---|---|---|---|---|---|---|
| | variety | | 2010 | | 2013 | |
| Thyme | Common | Frozen | 27.1 ± 0.0 | d* | nd | |
| | Wild | Frozen | 27.0 ± 0.2 | d | nd | |
| | Lemon | Frozen | 35.9 ± 0.1 | e | 12.1 ± 0.2 | c** |
| | | Dried | nd | | 12.3 ± 1.2 | c |
| Basil | Genovese | Frozen | 5.2 ± 0.0 | c | nd | |
| | Thai | Frozen | 2.9 ± 0.3 | a | nd | |
| | Cinnamon | Frozen | 4.4 ± 0.1 | b | 4.1 ± 0.1 | a |
| | | Dried | nd | | 5.3 ± 0.5 | b |

Data are means ±SD.
mg CAE/g fw, mg equivalent chlorogenic acid/g fresh plant; nd, not determined.
*The same letter in the column indicates that there is no significant difference between cultivars/varieties in 2010 (Duncan test, P = 0.05).
**The same letter in the column indicates that there is no significant difference between frozen and dried samples of lemon thyme and Cinnamon basil in 2013 (Duncan test, P = 0.05).

the same experimental field with the same soil composition and were collected on 19 July 2013. They were frozen or dried and left in a freezer or under dry conditions until the analyses 3 months later, which was approximately the same time lag as for the samples collected in 2010. The difference in TPH might thus have originated from other factors during the plant development, and most probably from temperature, photoperiod, and precipitation differences between the 2 growing seasons. Different studies have suggested that the environmental conditions during plant growth can explain the differences in TPH as well as the levels of individual phenolics (Flanigan and Niemeyer, 2014). It appears that the much lower precipitation in the growing season of 2010 (194 mm) as compared to 2013 (297 mm) provoked a more pronounced accumulation of TPH in this lemon thyme. Considering the Pearson correlation coefficients, the biomass for thyme correlates well with TPH (r = 0.762), while for basil there was no correlation seen (r = 0.102).

The data reported by Zheng and Wang (2001) are comparable to those of the present study for the frozen basil, but the frozen thyme in the present study showed much greater TPH contents. Phenolic acids (e.g., ferulic, gallic acids) are the main phenolics in thyme, followed by phenolic monoterpenes (e.g., thymol) and flavonoids (Jabri-Karoui et al., 2012). Despite the variations in the phenolic profiles of samples from different origins that have been ascribed to growing conditions, harvesting, and processing, rosmarinic acid was the predominant phenolic acid in both thyme and basil (Kwee and Niemeyer, 2011; Park, 2011; Martins et al., 2015).

Apart from environmental factors during growth, such differences can also be attributed to different genotypes (Čeh et al., 2007; Kacjan Maršić et al., 2011). Considering the samples from 2010, greater variability was seen for the frozen basil varieties (CV = 0.28) than for the frozen thyme species (CV = 0.17). Javanmardi et al. (2003) obtained different values across individual samples for dried basil (from 23 mg to 66 mg GAE/g dry weight (dw)). This again confirmed the high variability of TPH content across different species of *O. basilicum*. When the moisture content of the fresh basil samples in the present study was considered, the TPH would amount to 23.1 ± 2.1 mg GAE/g dw, which thus corresponds to the data of Javanmardi (2003), Kwee and Niemeyer (2011), and Flanigan and Niemeyer (2014).

Drying of lemon thyme collected in 2013 had no significant impact on TPH (Table 3). The dried Cinnamon basil compared to the frozen one had significantly higher TPH. This is quite interesting, bearing in mind that drying can reduce the TPH content due to oxidative deterioration of the phenolics. Such deterioration depends on the temperature of drying and the chemical composition of the ethanol extract. The greater TPH content of these basil ethanol extracts obtained from dried samples might also be due to the different sizes of the ground dried herb material; namely, the smaller the pieces, the larger the surface area, and the more efficient the extraction for

higher yields of the extracted compounds. The increased brittleness of plant tissue will also result in quicker cell wall breakdown during grinding and easier release of the phenolics into the extracting solvent. This effect is more evident for the more tender Cinnamon basil leaves compared to the more woody lemon thyme, as the cell tissue structure of the thyme will remain more intact. The influence of different methods of drying on the TPH content for thyme has revealed that after drying in the sun or in the shade, the TPH content increased in comparison to the fresh material (Hajimehdipoor et al., 2012). Higher TPH contents in dried thyme than in dried basil have also been reported after the 3 different drying treatments: air drying, freeze drying, and vacuum-oven drying (Hossain et al., 2010). Hossain et al. (2010) also indicated that there can be an inactivation of enzymes during drying, due to the decreased water activity. In samples that are not dried, Suhaj (2006) suggested that some antioxidants might be degraded by enzymatic reactions. Mansour (2016) reported that fresh common thyme had the highest contents of TPH and flavonoids, more than air-shade dried, air-sun dried, or oven dried common thyme. It has been also shown that the condition of extraction of phenolic compounds from fresh or lyophilized basil leaves influences TPH (Złotek et al., 2016).

In the ethanol extracts from lemon thyme, there were significantly higher levels (about 2-fold) of TFL, TFF, and TFDH than for the ethanol extracts from Cinnamon basil (Table 4). The data from lemon thyme reported by Pereira et al. (2013) were similar, with very low levels of flavonols. Meanwhile, Taie et al. (2010) reported that in basil, in addition to phenolic acids, flavones and flavonols are among the most represented phenolics. Considering the correlation analysis of the present study (Table 5),

**Table 4.** Flavonoid contents of ethanol extracts of frozen and dried lemon thyme and cinnamon basil plants from 2013.

| Herb | Sample | Total flavonoids (mg RUE/g fw) | | Total flavones/flavonols (mg QUE/g fw) | | Total flavanones/dihydroflavonols (mg NAE/g fw) | |
|---|---|---|---|---|---|---|---|
| Lemon | Frozen | 11.9 ± 0.3 | d* | 1.8 ± 0.1 | d* | 4.2 ± 0.2 | d* |
| thyme | Dried | 11.0 ± 1.0 | c | 1.0 ± 0.0 | b | 2.3 ± 0.1 | b |
| Cinnamon | Frozen | 5.2 ± 0.3 | a | 1.1 ± 0.0 | c | 2.5 ± 0.2 | c |
| basil | Dried | 6.2 ± 0.1 | b | 0.7 ± 0.0 | a | 1.6 ± 0.1 | a |

Data are means ± SD.
mg RUE/g fw, mg equivalent rutin/g fresh plant;
mg QUE/g fw, mg equivalent quercetin/g fresh plant;
mg NAE/g fw, mg equivalent naringenin/g fresh plant.
*The same letter in the column indicates that there is no significant difference between frozen and dried samples of lemon thyme and Cinnamon basil in 2013 (Duncan test, P = 0.05).

**Table 5.** Values of Pearson correlation coefficients for antioxidant potential as $IC_{50}$, and content of total phenolics and flavonoids in both frozen and dried lemon thyme and Cinnamon basil collected in 2013.

| Parameter | Pearson correlation coefficient | | | |
|---|---|---|---|---|
| | Total phenolics* | Total flavonoids** | Total flavones/flavonols*** | Total flavanones/dihydroflavonols**** |
| Antioxidant potential ($IC_{50}$) | -0.061 | 0.043 | 0.790 | 0.782 |
| Total phenolics | | 0.992 | 0.564 | 0.573 |
| Total flavonoids | | | 0.646 | 0.656 |
| Total flavones/flavonols | | | | 1.000 |

$IC_{50}$, concentration of total phenolics in the reaction mixture for 50% reduction of initial DPPH• (µg CAE/mL); lower $IC_{50}$ means higher antioxidant potential.
*mg CAE/g fw, mg equivalent chlorogenic acid/g fresh plant;
**mg RUE/g fw, mg equivalent rutin/g fresh plant;
***mg QUE/g fw, mg equivalent quercetin/g fresh plant;
****mg NAE/g fw, mg equivalent naringenin/g fresh plant.

TPH correlates well with total flavonoids - TFL (r = 0.99), but less so with total flavones/flavonols - TFF and total flavanones/dihydroflavonols - TFDH. However, the correlation between TFF and TFDH is very good (Table 5).

The air drying in the present study affected the TFL content of the ethanol extracts. The extract from dried lemon thyme had significantly lower TFL, while those from dried Cinnamon basil had higher TFL than the frozen ones (Table 4). As mentioned above, a higher yield might be ascribed to more effective ethanol extraction from the dried herb than from the fresh herb, as was also reported by Hajimehdipoor et al. (2012). On the other hand, the ethanol extracts from both of these dried herbs were significantly poorer in terms of TFF and TFDH than those from the frozen samples. It thus appears that TFF and TFDH are the subgroups of flavonoids that are particularly susceptible to deterioration, most probably due to oxidation during drying. In this respect, there was a loss of about 50% of the TFF and TFDH in the dried samples of lemon thyme and a loss of about 35% in the dried samples of Cinnamon basil compared to both of the frozen samples. This might indicate that the Cinnamon basil contains more stable flavonoids.

### 3.3. Antioxidant potential

The ethanol extracts from both the frozen lemon thyme and the Cinnamon basil collected in 2013 showed almost the same levels of reduction of the DPPH$^{\bullet}$ radicals (Figure 2). The ethanol extracts from the frozen herbs had appreciably lower AOP than those from the dried herbs, although the dried lemon thyme showed comparable TPH and TFL contents, and the dried Cinnamon basil had only slightly lower TPH and TFL contents, compared to their frozen counterparts (Tables 3 and 4). The present data are in agreement with those of Zheng and Wang (2001) and Martins et al. (2015), but they differ from those of Lagouri and Nisteropoulou (2009).

These data can be explained considering that for the DPPH$^{\bullet}$ radical scavenging activity the electron and/or hydrogen atom donating ability of compounds is important, and that this does not always correlate with the phenolics redox potential (Huang et al., 2005; Bounatirou et al., 2007). The Folin–Ciocalteu method is based on the transfer of electrons from compounds in the reaction mixture with a certain redox potential to reduce the phosphomolybdates and phosphotungstic acid in the reagent. This behavior can be confirmed by the lack of correlation between AOP and TPH (r = -0.061) and TFL (r = 0.043), as shown in Table 5 for the present study, and also in some other studies where poor or even no correlation was seen (Hinneburg et al., 2006; Mata et al., 2007).

The AOP of extracts depends on their composition, and the individual phenolics profile can have greater influence on AOP than the content of total phenolics (Kwee and Niemeyer, 2011; Teofilović et al., 2017). Moreover, the AOP also depends on the assay conditions (i.e. type, composition) (Wanasundara and Shahidi, 1998). The AOP in the present study was estimated in ethanol and in the Folin–Ciocalteu assay in aqueous solution at basic pH. It has been shown that aqueous solution at basic pH supports ionization of phenolics and consequently, depending on the structure and composition of the compounds, the reactivity is higher (Abramovič et al., 2017).

Higher AOP of the dried plants can also be explained by the effective release of phenolics that were bound to the cell structural elements (Hajimehdipoor et al., 2012). High Pearson correlation coefficients observed for the correlations between $IC_{50}$ and TFF (r = 0.79) or TFDH (r = 0.78) (Table 5) mean that the dried herbs had higher AOP (i.e. lower $IC_{50}$), less TFF, and less TFDH than the frozen herbs. Despite the comparable AOP, the samples of Cinnamon basil had lower TFF and lower TFDH than lemon thyme. This would suggest that neither TFF nor TFDH are the key compounds responsible for these comparable AOP levels. It could be that other phenolics that are more stable during drying contribute to the

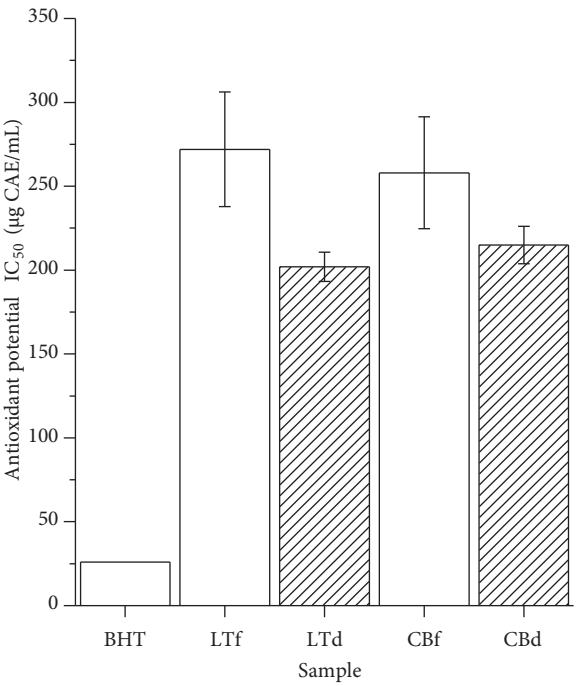


**Figure 2.** Antioxidant potential $IC_{50}$ of the ethanol extracts of lemon thyme and Cinnamon basil grown in 2013, in comparison to the synthetic antioxidant t-butylhydroxytoluene (BHT). $IC_{50}$, concentration of total phenolics in reaction mixture for 50% reduction of initial DPPH$^{\bullet}$; LTf, lemon thyme frozen; LTd, lemon thyme dried; CBf, Cinnamon basil frozen; CBd, Cinnamon basil dried.

AOP. Indeed, for basil and thyme, rosmarinic acid with 4 hydroxyl groups (2 pairs of –OH, each one with the catechol structure) is the predominant phenolic and the most important antioxidant (Park, 2011), with thyme richer than basil (Lagouri and Nisteropolou, 2009). Increased levels of rosmarinic acid in thyme and basil after drying, with higher levels for thyme and higher AOP for dried samples than for fresh samples, were also shown by Hossain et al. (2010).

In comparison to BHT in the present study, the samples analyzed had much lower AOP. This means that to obtain the same effect as BHT, they should be added at higher concentrations. However, contrary results have been reported for BHT in the literature (Mata et al., 2007; Lagouri and Nisteropoulou, 2009).

**3.4. Antibacterial activity**

The vacuum dried ethanol extracts of lemon thyme and Cinnamon basil were used to determine their antibacterial activities against 4 bacteria, with each tested as 6 strains. All of the MICs across the strains of *S. aureus*, *L. monocytogenes*, *C. jejuni*, and VTEC are relatively high (Table 6). In 2 cases, the MICs were not determined because even the highest concentration tested did not inhibit the growth of any of the VTEC strains tested.

The lowest mean MICs were seen against *C. jejuni*. However, there were relatively large differences in the sensitivities of the strains tested. In general, the lemon thyme ethanol extracts showed greater activities against the gram-positive bacteria than the Cinnamon basil ethanol extracts. However, none of these ethanol extracts were particularly efficient against the gram-negative VTEC strains (Table 6). The MICs obtained here for the lemon thyme were high, while the ethanol extracts from frozen and dried Cinnamon basil did not inhibit the growth of these VTEC strains in the concentration range tested.

It is still not understood how the different chemical compositions of such extracts are reflected in their antimicrobial activities (Gyawali and Ibrahim, 2014). Here, to determine at least which group of phenolics, as TFL, TFF, and TFDH, their contents were correlated with the MICs (Table 7). TFL are present in all of the ethanol extracts, and among these, TFDH prevail (Table 4). The highest TFDH content was seen for the frozen lemon thyme ethanol extract, which in general showed the lowest MICs against different microbial targets. We can assume that the compounds in this TFDH group have an important influence on the antimicrobial activity. Similar data were obtained in some of our previous studies (Katalinić et al., 2010; Trošt et al., 2016). However, the highest correlation was seen between the MIC for *S. aureus* and the TPH and TFL, followed by the correlation between the MIC for *L. monocytogenes* and the TFF and TFDH. For the MIC of *C. jejuni*, higher correlations were seen for TPH and TFL, rather than for TFF and TFDH. Due to the lack of efficiency of these ethanol extracts against VTEC, no correlation study was possible for this gram-negative bacterium.

The phenolic –OH groups can disrupt cell membrane structure and cause leakage of cellular components and as such can have important roles in their antimicrobial actions (Gyawali and Ibrahim, 2014). Furthermore, they can alter cell metabolism by binding to enzyme active sites and/or reducing proton gradients across membranes, which might also vary between different types of microorganisms.

In conclusion, 3 species of thyme and 3 varieties of basil that were all organically grown were used to determine the biomass per plant and the TPH. Furthermore, for the ethanol extracts of lemon thyme and Cinnamon basil, the TFL, TFF, TFDH, AOP, and antimicrobial activities were determined. The extracts of frozen and dried lemon thyme contained more TPH, TFL, TFF, and TFDH than those of Cinnamon basil. The air drying did not affect the TPH for lemon thyme, but for the dried Cinnamon basil the TPH was higher, and TFF and TFDH decreased considerably. The ethanol extracts of these herbs showed AOP that was much lower in comparison to the synthetic antioxidant

**Table 6.** Antimicrobial activities of ethanol extracts of frozen and dried lemon thyme and Cinnamon basil plants from 2013 against the 4 foodborne microorganisms (each tested on 6 strains).

| Herb | Sample | Minimum inhibitory concentration (µg CAE/mL) | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *L. monocytogenes* | *C. jejuni* | Verotoxin *E. coli* |
| Lemon thyme | Frozen | 621 ± 217 | 1064 ± 0 | 288 ± 252 | 3900 ± 868 |
| | Dried | 490 ± 172 | 1190 ± 413 | 306 ± 286 | 6158 ± 1371 |
| Cinnamon basil | Frozen | 954 ± 209 | 1017 ± 394 | 588 ± 272 | >3050 ± 0 |
| | Dried | 624 ± 160 | 1953 ± 713 | 223 ± 61 | >5206 ± 0 |

Data are means ± SD.
µg CAE/mL, µg equivalent chlorogenic acid/mL growth medium for total phenols.

**Table 7.** Values of Pearson correlation coefficients between the antioxidant potential as $IC_{50}$ and the content of total phenolics and flavonoids and the antimicrobial activities of the respective ethanol extracts prepared from both frozen and dried lemon thyme and Cinnamon basil collected in 2013.

| Parameter | Pearson correlation coefficients | | |
|---|---|---|---|
| | MIC *S. aureus* | MIC *L. monocytogenes* | MIC *C. jejuni* |
| Antioxidant potential ($IC_{50}$) | 0.575 | -0.560 | 0.434 |
| Total phenolics* | -0.758 | -0.369 | -0.487 |
| Total flavonoids** | -0.725 | -0.373 | -0.499 |
| Total flavones/flavonols*** | 0.006 | -0.682 | 0.051 |
| Total flavanones/dihydroflavonols**** | -0.011 | -0.670 | 0.032 |

MIC, minimum inhibitory concentration (µg CAE/mL).
$IC_{50}$, concentration of total phenolics in reaction mixture for 50% reduction of initial DPPH• (µg CAE/mL); lower $IC_{50}$ means higher antioxidant potential.
*mg CAE/g fw, mg equivalent chlorogenic acid/g fresh plant;
**mg RUE/g fw, mg equivalent rutin/g fresh plant;
***mg QUE/g fw, mg equivalent quercetin/g fresh plant;
****mg NAE/g fw, mg equivalent naringenin/g fresh plant.

BHT and those from the dried herbs had higher AOP than the ones from the frozen herbs. The MICs of the ethanol extracts varied mostly according to the target bacteria, although they were poor antimicrobial agents, especially against gram-negative VTEC. However, the ethanol extracts from lemon thyme were more effective inhibitors of *C. jejuni*, *S. aureus*, and *L. monocytogenes* growth than the ethanol extracts from Cinnamon basil, potentially due to the different chemical compositions.

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