Comparative study on the chemical composition of laurel (Laurus nobilis L.) leaves from Greece and Georgia and the antibacterial activity of their essential oil

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ABSTRACT

Laurel (Laurus nobilis L.) is a plant species from Lauraceae family, and is native to the Mediterranean region. The goal of this study was to compare chemical composition of laurel leaves and antibacterial activity of its essential oil (EO) from wild-grown trees in Greece and Georgia. The laurel leaves from the two native habitats had different concentrations of phenolic acids. Of the conjugated flavonols and flavones, kaempferol (1981.3 μg/g) and apigenin (1433.6 μg/g) were the major representatives in the leaves from Greece, while luteolin (839.1 μg/g) and kaempferol (688.1 μg/g) were the major ones in the leaves from Georgia, respectively. The EO content was 1.42% and 4.54% in the leaves from Greece and Georgia, respectively. The main EO constituents of the Greek laurel plants were 1,8-cineole (30.8%), α-terpinyl acetate (14.9%), α-terpineol (8.0%), sabinene (7.9%), and terpine-4-ol (6.0%). The main EO constituents of the Georgian laurel plants were 1,8-cineole (29.2%), α-terpinyl acetate (22.6%), sabinene (12.2%), and methyleugenol (8.1%). The EO antimicrobial activities against 20 microorganisms were determined. Among the Gram-positive bacteria, the Enterococcus faecalis strain was the most sensitive, followed by Staphylococcus aureus. Among the Candida species, C. albicans ATCC 10231 was the most sensitive to the laurel leaf EOs.

1. Introduction

Laurel (Laurus nobilis L.) fam. Lauraceae is a shrub native to the Mediterranean region and cultivated in a number of countries in Asia, Europe, and the Americas as a spice, or used as ornamental plant (Parthasarathy et al., 2008). Laurel leaves, also known as bay leaves, are a major spice, which has been used traditionally as an ingredient for improving food flavor and taste. Naturally occurring biologically active compounds in leaves include terpenes, terpene derivatives, polyphenols, alkaloids, minerals, vitamins (Caputo et al., 2017; Chahal et al., 2017; Parthasarathy et al., 2008).

The essential oil (EO) content of laurel leaves has been reported to range between 0.2% and 4.3% depending on the location, harvesting time, and EO extraction type and conditions (e.g. hydrodistillation or steam distillation) (Abu-Dahab et al., 2014; Bahmanzadegan et al., 2015; El et al., 2014; Fidan et al., 2019; Shokoohinna et al., 2014; Vasundhara et al., 2016). Previous research has shown that up to 270 EO constituents may be found in laurel leaves, the major ones being 1,8-cineole (22–56%), linalool (0.9–26.9%), α-terpinyl acetate (4.5–18.2%), α-pinene (2.2–15.9%), β-pinene (1.9–15.3%), sabine (4.5–12.7%), α-terpineol (0.9–12.0%), terpineol-4 (0.9–4.1%) (Abu-Dahab et al., 2014; Bahmanzadegan et al., 2015; Chahal et al., 2017; El et al., 2014; Fidan et al., 2019; Goudjil et al., 2015; Shokoohinna et al., 2014; Vasundhara et al., 2016). Similarly to other EO-containing plants, the composition of laurel leaf EO has been shown to vary significantly as a function of the environment, genotype, and the type and duration of the distillation process (Abu-Dahab et al., 2014; Bahmanzadegan et al., 2015; Chahal et al., 2017; El et al., 2014; Fidan et al., 2019; Goudjil et al., 2015;
Shokoohinia et al., 2014; Vasundhara et al., 2016). A recent retrospective on *L. nobilis* chemistry and biological activities of its EO was published by Chahal et al. (2017). In addition, laurel leaf EO has exhibited antimicrobial and antioxidant activities (Bahmanzadegan et al., 2015; Caputo et al., 2017; Dias et al., 2014; El et al., 2014; Fidan et al., 2019).

The main laurel leaf suppliers on the international market are Turkey, Portugal, Spain, and Iran, although native populations are rare and scattered around the Mediterranean, they could potentially be used for development of new varieties. Besides, wild-collected *L. nobilis* may have better nutritional value than cultivated laurel (Dias et al., 2014). The goal of this study was to compare the chemical composition of the laurel leaves from wild-grown trees found in two different European countries (Greece and Georgia) and assess the antimicrobial activity of their EOs against pathogenic and spoilage microorganisms.

2. Materials and methods

2.1. Chemicals

The following reagents and chemicals were used in this study: polyphenols, 1,8-cineole, and anhydrous sodium sulfate from Sigma-Aldrich; Nutritional Agar (NA); Sabouraud Dextrose Agar with chloramphenicol; 2.1. Chemicals against pathogenic and spoilage microorganisms. (Greece and Georgia) and assess the antimicrobial activity of their EOs leaves from wild-grown trees found in two different European countries (Greece and Georgia) and assess the antimicrobial activity of their EOs against pathogenic and spoilage microorganisms.

2.2. Plant material

Leaves from *L. nobilis* L. were collected only from wild growing trees, from one location per country. Subsamples were generated from 10 different trees per location by sampling leaves at different height and exposure within a tree. The leaves were harvested at the end of vegetation, during the formation of fruits. The leaves were collected in October 2016 from the Athos peninsula on a land belonging to the Bulgarian monastery (North Greece, at 160 m elev., 40°09′26″N and 24°19′35″E) and in December 2016 from the province of Meria (West Georgia, at 200 m elev., 41°56′27″N 41°53′45″E).

The collected samples were identified as *Laurus nobilis* L. by Dr. Ivanka Dimitrova at the University of Plovdiv “Paisii Hilendarski” in Plovdiv, Bulgaria. The moisture (39.7 ± 0.35%) for leaves from Greece and 29.5 ± 0.35% for leaves from Georgia) was determined after drying at 105°C to constant weight (The State Pharmacopoeia of the USSR, 1990). The samples were analyzed for polyphenols and EO content and the values were presented on an absolute dry weight basis.

2.3. Determination of polyphenols

Sample preparation and HPLC analyses were carried out according to Marchev et al. (2011).

HPLC Analyses: Quantitative and qualitative measurements of phenolic acids and flavonoids were conducted using Waters 1525 Binary Pump HPLC systems (Waters, Milford, MA, USA) that had a Waters 2484 dual Absorbance Detector (Waters, Milford, MA, USA) and Supelco Discovery HS C18 column (5 μm, 25 cm × 4.6 mm) these operated under Breeze 3.30 software control.

Analyses of phenolic acids and flavonoids were carried out according to Ivanov et al. (2014) and Marchev et al. (2011).

2.4. Isolation of the essential oil (EO)

The EO was extracted via 3-h hydrodistillation of 100 g laurel leaves using Cleveenger-type glass apparatus of the British Pharmacopoeia, modified by Balinova and Diakov (1974). The obtained EO was dried with anhydrous sodium sulfate, and placed in dark vials at 4 °C until gas chromatographic (GC) analysis.

2.4.1. Gas chromatographic analyses for the chemical composition of essential oil

A GC analysis was carried out using an Agilent 7890A gas chromatograph, HP-5 column MS (30 m × 250 μm x 0.25 μm), temperature: 35 °C/3 min, 5 °C/min to 250 °C for 3 min, 49 min in total, helium as carrier gas, 1 ml/min constant speed, 30:1 split ratio. A gas chromatography–mass spectrometric (GC/MS) analysis was performed on an Agilent 5975C mass spectrometer, helium as carrier gas, column and temperature the same as in the GC analysis. The identification of the chemical constituents was made by comparison to their retention time and library data (Adams, 2007; NIST 08 database; own libraries). Components were listed according to their retention ( Kovat's) indices, calculated using a standard calibration mixture of C₈ - C₉₀ n-alkanes in n-hexane. Compound concentration was computed as percentage of the total ion current (TIC).

2.5. Antimicrobial activity of EOs

The antimicrobial effects of the EOs were evaluated against Gram-positive bacteria Bacillus cereus ATCC 11778, Enterococcus faecalis (clinical isolate), Enterococcus faecium (clinical isolate), two strains of Staphylococcus aureus (ATCC 6538 and one food spoilage isolate), and Listeria monocytogenes NCTC 11994; Gram-negative bacteria: Escherichia coli ATCC 25922, Klebsiella pneumoniae (clinical isolate), two strains of Salmonella abortion (ATCC 6017 and one clinical isolate), Shigella flexneri (clinical isolate), two strains of Pseudomonas aeruginosa (ATCC 27853 and one clinical isolate), and a food spoilage isolate of Pseudomonas fluorescens. Proteus mirabilis (clinical isolate), and Proteus vulgaris (clinical isolate); yeasts: two strains of Candida albicans (ATCC 10231 and one clinical isolate), C. glabrata (clinical isolate) and C. tropicalis (clinical isolate). The clinical isolates from bacteria were kindly provided by the Department of Microbiology and Immunology at the Medical University of Plovdiv, and the yeast isolates were kindly provided by the National Referent Laboratory of Mycology at the National Center of Infectious and Parasitic Diseases in Sofia, Bulgaria.

All strains were deposited in the Microbial Culture Collection of the Department of Biochemistry and Microbiology at Paisii Hilendarski University of Plovdiv Bulgaria.

The antibacterial activity of the EOs was assessed according to the Clinical Laboratory Standard Institute (CLSI) reference method for antimicrobial disk susceptibility tests (CLSI, M2-A9 2006; CLS, M7-A7 2006). The antifungal activity of the EOs was assessed according to the CLSI reference method for antifungal disk diffusion susceptibility testing of yeast (CLSI, M2-A3 2008; CLSI, M44-A2 2009). The used filter paper discs (Whatman N21) in disc diffusion test were prepared by soaking with 10 μL of the tested essential oil samples. 1,8-Cineole represents about 30% of the total oil content and the filter paper discs were prepared by soaking with 3 μL of the 1,8-cineole. The used positive controls of reference antibiotic discs Ciprofloxacin (5 μg/disc) and antymycotic Fluconazole (25 μg/disc) were purchased by HiMedia (India). The antimicrobial activity of the EOs and 1,8-cineole determined by disc diffusion tests was expressed as inhibitory zone (IZ) in mm; these zones were measured to the nearest millimeter using an antibiotic zone scale. The antimicrobial activities of CPH and FLC were also determined as positive controls.

2.6. Statistical analysis

All experiments were performed at least three times. All data were presented as mean ± standard error of the mean. Statistical significance was assessed by either Student’s-test or one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison Differences between means were considered statistically significant if p > 0.05.
3. Results and discussion

3.1. Polyphenol composition

The polyphenol content is shown in Table 1.

In this study, twelve phenolic acids were quantified in the laurel leaves (Table 1). Overall, the results showed significant differences in the free and conjugated phenolic acid content, which was determined after acid hydrolysis. In the laurel leaves from Greece, in the hydroxycinnamic acid derivatives, sinapic (607.7 μg/g), caffeic (586.1 μg/g), and ferulic (300.1 μg/g) acids were the major free phenolic acids, while ferulic (2193.0 μg/g), sinapic (560.4 μg/g), and cinnamic (486.2 μg/g) acids were the major conjugated acids. The major free hydroxybenzoic acid derivatives were syringic (242.0 μg/g) and salicylic (207.3 μg/g) acids, while vanillic acid (83.9 μg/g) was the major conjugated acid. Kaempferol (1981.3 μg/g) and apigenin (1433.6 μg/g) were the major representatives of the group of conjugated flavonols and flavans, respectively. In the group of quercetin glycosides only the free form of rutin and hyperoside in the Greek leaves was identified. In the laurel leaves from Georgia, p-coumaric acid (45.3 μg/g) was the major free phenolic acid in the group of the hydroxycinnamic acid derivatives, while sinapic (1513.9 μg/g), caffeic (789.3 μg/g), and cinnamic (513.4 μg/g) acids were the major conjugated acids. In the group of the hydroxybenzoic acid derivatives the dominant was syringic acid (789.1 μg/g). Luteolin (839.1 μg/g) and kaempferol (688.1 μg/g) were the major constituents of the group of conjugated flavonols and flavans, respectively. The derivatives of the group of conjugated cinnamic acids were the dominant compounds in the Georgian leaves, while the level of flavonols and flavans was lower (Table 1).

The observed differences in the polyphenol composition of the samples from the two countries can be explained by the different environmental conditions at the two collection sites. Mount Athos on the Athos peninsula (Greece) is on the Aegean coast, and the village of Meria in Western Georgia is near the Black Sea. The long-term average annual precipitation at the sampling site in Greece (Hilandar on Mount Athos) is 474 mm. At that location, the average temperature in July is 25 °C, while the one in January is 8 °C. Also, most of the precipitation occurs in December, with an average of 80 mm of rain. On the other hand, the climate at the sampling location in Western Georgia, around the village of Meria, is warm, humid, mild, and temperate. The weather station at Ozurgeti, Georgia (around 10 km from the collection site at Meria) has long-term average annual precipitation of 1981 mm, with average temperature in August of 22.7 °C, and average temperature in January of 5.0 °C. In general, the results reported here showed that climatic differences in both regions caused significant differences in leaf polyphenols. Therefore, our results are in agreement with previous literature reports on other plants, which are cultivated in different geographic regions (Dragovic-Uzelac et al., 2007; Franquet-Griell et al., 2012).

### Table 1. Polyphenol content of laurel leaves from Greece and Georgia (mean ± SD).

| N° | Compounds                        | RT, min | Content, μg/g dw | Leaves from Greece |                     | Leaves from Georgia |                     |
|----|----------------------------------|---------|-----------------|--------------------|---------------------|---------------------|---------------------|
|    |                                  |         |                 | Free               | Conjugated         | Free                | Conjugated         |
| 1  | Rosmarinic acid                  | 6.54    | 47.5 ± 0.08     | *                  | -                   | -                   | -                   |
| 2  | Chlorogenic acid                 | 33.13   | 243.9 ± 0.36    | -                  | -                   | 48.1 ± 0.04         |                     |
| 3  | Caffeic acid                     | 38.59   | 56.8 ± 0.09     | 31.4 ± 0.05        | 789.3 ± 0.81        |                     |                     |
| 4  | p-Coumaric acid                  | 46.59   | 246.6 ± 0.35    | 45.3 ± 0.06        | 375.9 ± 0.36        |                     |                     |
| 5  | Sinapic acid                     | 47.14   | 560.4 ± 0.52    | -                  | 1513.9 ± 1.22       |                     |                     |
| 6  | Ferulic acid                     | 47.69   | 2193.0 ± 2.11   | 29.4 ± 0.04        | 70.4 ± 0.09         |                     |                     |
| 7  | Cinnamic acid                    | 53.41   | 486.2 ± 0.51    | 22.7 ± 0.04        | 513.4 ± 0.71        |                     |                     |
| 8  | Gallic acid                      | 9.47    | 24.8 ± 0.03     | -                  | -                   | -                   | -                   |
| 9  | Protocatechuic acid              | 19.19   | 17.2 ± 0.02     | 13.3 ± 0.02        | 68.6 ± 0.07         |                     |                     |
| 10 | Salicylic acid                   | 28.87   | 41.7 ± 0.04     | -                  | 29.4 ± 0.03         |                     |                     |
| 11 | Vanillic acid                    | 37.84   | 37.0 ± 0.04     | -                  | -                   | -                   | -                   |
| 12 | Syringic acid                    | 39.86   | 19.6 ± 0.01     | 5.9 ± 0.0          | 789.1 ± 0.72        |                     |                     |
| 13 | Myricetin                        | 15.63   | 124.5 ± 0.11    | 75.2 ± 0.08        | 75.5 ± 0.08         | 47.2 ± 0.04         |                     |
| 14 | Quercetin                        | 21.46   | 42.3 ± 0.04     | 65.3 ± 0.07        | 44.9 ± 0.05         |                     |                     |
| 15 | Kaempferol                       | 26.78   | 1981.3 ± 2.00   | 250.7 ± 0.24       | 688.1 ± 0.79        |                     |                     |
| 16 | Luteolin                         | 23.89   | 388.6 ± 0.36    | 59.0 ± 0.06        | 839.1 ± 0.84        |                     |                     |
| 17 | Apigenin                         | 27.93   | 1433.6 ± 1.12   | 161.7 ± 0.15       | 262.7 ± 0.25        |                     |                     |
| 18 | Hesperetin                       | 16.09   | 116.4 ± 0.11    | -                  | -                   | 31.2 ± 0.03         |                     |
| Quercetin glycosides
| 19 | Rutin                            | 7.42    | 217.4 ± 0.20    | -                  | -                   | -                   | -                   |
| 20 | Hyperoside                       | 8.98    | 141.8 ± 0.13    | -                  | -                   | -                   | -                   |

* Compound not found.
Terpinen-4-ol OM 17.09 1179 6.0

11. Terpinen-4-ol OM 17.09 1179 6.0

12. α-Terpinol OM 17.56 1189 8.0 0.10 1.7

13. Borrel acetate OM 20.01 1269 1.2 0.05 0.09

14. α-Terpinyl acetate OM 21.87 1333 14.9 0.0 0.8

15. Eugenol PP 21.98 1363 2.7 0.0 0.0

16. Methyleneugenol PP 23.17 1371 3.6 0.0 0.0

17. β-Elemene SH 24.20 1390 29.2 0.0 0.0

18. β-Caryophyllene SH 24.49 1429 0.4 0.0 0.0

19. Germacrene D SH 25.18 1484 0.3 0.0 0.0

20. Elemicin SH 26.10 1522 3.8 0.0 0.0

21. Caryophyllene oxide OS 27.49 1574 1.8 0.0 0.0

22. Spathulenol OS 27.61 1619 0.4 0.0 0.0

23. β-Eudesmol OS 28.05 1642 0.7 0.0 0.0

24. n-Heptadecane AH 29.27 1700 0.2 0.0 0.0

25. n-Heneicosane AH 32.50 2100 0.6 0.0 0.0

26. Phytool D 32.86 2105 1.5 0.0 0.0

27. n-Doicosane AH 33.79 2200 0.7 0.0 0.0

28. n-Tricosane AH 35.00 2300 0.4 0.0 0.0

29. n-Tetracosane AH 36.13 2400 0.3 0.0 0.0

30. n-Pentacosane AH 38.10 2500 0.5 0.0 0.0

31. n-Hexacosane AH 39.88 2600 0.8 0.0 0.0

32. n-Heptacosane AH 41.72 2700 0.9 0.0 0.0

33. n-Octacosane AH 44.40 2800 0.3 0.0 0.0

34. Squalene T 45.02 2817 0.9 0.0 0.0

Aliphatic hydrocarbons (AH),% 4.79 1.66

Monoterpen hydrocarbons (MH),% 22.10 25.50

Oxygenated monoterpenes (OM),% 61.46 60.40

Sesquiterpene hydrocarbons (SH),% 0.73 1.71

Oxygenated sesquiterpenes (OS),% 2.18 0.39

Diterpenes (D),% 1.49 0.11

Triterpenes (T),% 0.90 0.30

Phenyl propanoids (PP),% 6.37 9.93

1 RI – retention (Kovat’s) index.

TIC – total ion current; All data are presented as mean value ± standard deviation (n = 3).

nd – below 0.05% of TIC or not detected.
reported previously. Previous reports showed that the laurel leaf EOs produced from different regions was rich in 1,8-cineole (5.7–7.9%), α-terpinyl acetate (5.9–9.2%), limonene (3.1–12.5%), and α-pinene (3.0–7.4%), and β-pinene (1.6–13.4%) (Abu-Dahab et al., 2014; Bahmanzadegan et al., 2015; El et al., 2014; Fidan et al., 2019; Goudjil et al., 2015; Shokoohinna et al., 2014; Vasundhara et al., 2016).

Oxigenated monoterpenes (1,8-cineole, α-terpinyl acetate, α-terpineol, terpinen-4-ol) were the dominant group in the Greek laurel EO, followed by monoterpenes hydrocarbons (sabinene, α-pinene, β-pinene, γ-terpinene), phenyl propanoids (methyl eugenol) and aliphatic hydrocarbons. Oxigenated monoterpenes were also the dominant group in the Georgian laurel EO, followed by monoterpenes hydrocarbons and phenyl propanoids.

3.3. Antimicrobial activity of the essential oils (EOs)

In this study, the laurel leaf EO samples demonstrated antimicrobial activity against all of the Gram-positive bacteria and yeast tested (Table 3).

Among the Gram-positive bacteria, the strain belonging to E. faecalis was the most sensitive, followed by S. aureus ATCC 6538, whereas L. monocytogenes NCTC 11994 was the most resistant. Among the species belonging to the Candida genus, C. albicans was the most sensitive and C. glabrata was the most resistant to the tested laurel leaf EOs. Also, P. aeruginosa and P. fluorescens were resistant to the tested EO samples. S. flexneri and K. pneumoniae were resistant to the EOs. Overall, the Gram-positive bacteria and yeasts were more sensitive to the tested EOs in comparison with the Gram-negative bacteria, which is in agreement with previous reports (Griffin et al., 1999).

In general, the laurel leaf EO from Georgia demonstrated weaker inhibitory activity. The difference in the antimicrobial activities of the tested EO samples was most probably due to the differences in their chemical composition. The content of oxygenated monoterpenes in the two oils was different; e.g. 1,8 cineole, α-terpinyl acetate, α-terpinyl acetate, terpinen-4-ol, etc., and the presence of these compounds may explain the antimicrobial activity of EOs (Griffin et al., 1999). Antimicrobial activity of 1,8-cineole, which is the most abundant component of both laurel EOs was also studied. Pure 1,8-cineole demonstrated weaker antimicrobial activity in comparison with EOs, which means that antimicrobial activity of laurel EOs could not be attributed only to the dominant compound but the additional effect of some minor compounds as well as synergistic effects may have played a role.

The antimicrobial activity of the tested EOs in this study was comparable to the activity of the antibacterial antibiotic ciprofloxacin and antimycotic flunoxazole with the exception of the EO activity against Pseudomonas spp. The different antimicrobial resistance of both types of bacteria was probably due to the different structure and chemical composition of the cell wall of Gram-positive and Gram-negative bacteria. Indeed, the so-called “external membrane”, which is typical for Gram-negative bacteria, could prevent or delay the diffusion of the EO extract from the nutritive medium through the cell wall and membrane into the cytoplasm.

The EOs also demonstrated antimicrobial activity against three species of medically important yeasts belonging to the Candida genus. Overall, NAC species C. glabrata and C. tropicalis were more resistant in comparison with C. albicans.

The results from this study confirmed published data on the antimicrobial activities of laurel EOs (El et al., 2014; Goudjil et al., 2015). However, in some of the previous reports, the laurel leaf EO was isolated following different methods, and the plant material originated from other locations with different environmental conditions.

The results from this research were utilized by members of the author’s team for the development of documentation needed for steam distillation processing equipment and facility of laurel leaves from wild

### Table 3. Antimicrobial activity of laurel leaf essential oils from wild-grown trees in Greece and Georgia (mean ± SD).

| Test microorganisms   | Inhibitory zone diameter (IZ), mm | Antibiotics |
|-----------------------|-----------------------------------|-------------|
|                       | Laurel leaf essential oil from Greece 10 μl/disc | Laurel leaf essential oil from Georgia 10 μl/disc | 1,8-cineole 3 μl/disc | Ciprofloxacin 3 μl/disc |
| Gram-positive bacteria |                                   |             |                |
| B. cereus ATCC 11778  | 18.0 ± 0.18                       | 12.0 ± 0.13 | 10.2 ± 0.30    | 28.0 ± 0.27 |
| E. faecalis (clinical isolate) | 52.0 ± 0.53                      | 11.5 ± 0.13 | 10.0 ± 0.11    | 26.0 ± 0.26 |
| E. faecium (clinical isolate) | 20.0 ± 0.19                      | 12.0 ± 0.13 | 10.0 ± 0.11    | n/a          |
| S. aureus ATCC 6538   | 40.0 ± 0.38                       | 12.5 ± 0.13 | 10.5 ± 0.13    | 30.0 ± 0.29  |
| S. aureus (clinical isolate) | 19.0 ± 0.19                      | 15.0 ± 0.16 | 12.5 ± 0.13    | 30.0 ± 0.29  |
| L. monocytogenes NCTC 11994 | 12.0 ± 0.13                      | 10.0 ± 0.10 | 9.5 ± 0.11     | 18.0 ± 0.17  |
| Gram-negative bacteria |                                   |             |                |
| E. coli ATCC 25922    | 15.6 ± 0.17                       | n/a         | 8.4 ± 0.08     | 21.5 ± 0.20  |
| K. pneumoniae (clinical isolate) | 10.0 ± 0.11                      | n/a         | 13.0 ± 0.13    |
| S. aborty ATCC 6017   | 14.0 ± 0.14                       | 9.5 ± 0.11  | 8.4 ± 0.08     | 12.5 ± 0.13  |
| S. aborty (clinical isolate) | 14.0 ± 0.14                      | 9.0 ± 0.10  | 8.4 ± 0.08     | 18.5 ± 0.20  |
| S. flexneri (clinical isolate) | 14.0 ± 0.14                      | n/a         | 16.5 ± 0.14    |
| P. aeruginosa ATCC 27853 | n/a                             | n/a         | 15.0 ± 0.14    |
| P. aeruginosa (clinical isolate) | n/a                             | n/a         | 12.0 ± 0.13    |
| P. fluorescens (clinical isolate) | n/a                             | n/a         | 16.8 ± 0.17    |
| P. mirabilis (clinical isolate) | 11.0 ± 0.12                      | n/a         | 12.0 ± 0.13    |
| P. vulgaris (clinical isolate) | 12.0 ± 0.13                      | 8.5 ± 0.08  | 8.2 ± 0.08     | 12.5 ± 0.13  |
| Yeasts                |                                   |             |                |
| C. albicans ATCC 10231 | 24.8 ± 0.24                       | 16.3 ± 0.14 | 14.2 ± 0.13    | 21.5 ± 0.21  |
| C. albicans (clinical isolate) | 21.0 ± 0.21                      | 16.0 ± 0.14 | 14.1 ± 0.11    | 22.5 ± 0.22  |
| C. glabrata (clinical isolate) | 15.0 ± 0.14                      | 10.0 ± 0.10 | 9.1 ± 0.11     | 21.5 ± 0.20  |
| C. tropicalis (clinical isolate) | 16.6 ± 0.15                      | 12.0 ± 0.13 | 10.1 ± 0.11    | 16.5 ± 0.16  |

* n/a – no inhibition detected.
trees around the Bulgarian monastery in Mount Athos, Greece. Consequently, in 2019, laurel leaves EO was isolated at this processing facility. The resulting EO is currently being evaluated for its utilization in various food, cosmetic and other consumer products, subject to further research by the research team. In addition, members of the research team are developing documentation for the industrial processing of the wild grown laurel around the village of Meria, Georgia.

4. Conclusions

This study found significant dissimilarities in the chemical profile of laurel leaf EOs from Greece and Georgia. Derivates of hydroxycinnamic and hydroxybenzoic acids, flavonols and flavones dominated in the laurel leaves from Greece, whereas the dominant compounds in the Georgian leaves were the group of conjugated cinnamic acids. The main group of constituents in the EOs from Greek and Georgian laurel leaves was composed of the oxygen monoterpenes, 1,8-cineole, α-terpinyl acetate, α-terpineol, and terpinen-4-ol. The EOs demonstrated antimicrobial activity against pathogenic and spoilage microorganisms. Laurel plants from Greece and Georgia show promise as a new source of laurel leaves for the international spice market.

Declarations

Author contribution statement

Galina Stefanova, Tanya Girova, Velizar Gochev, Magdalena Stoyanova, Zhana Petkova, Valtcho D. Zheliazkov: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Albena Stoyanova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data Availability Statement

Data included in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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