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# Volatile Flavor Constituents, Total Phenols and Antioxidant Capacity of Chamomile from Different Areas of Greece

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*Abstract* – **The objective of this study was to identify the volatile constituents of chamomile (dried chamomile flower heads and chamomile infusions) and to determine the total phenols and the antioxidant capacity in infusions and methanol extracts of chamomile flowers from eight areas of Greece (Rodopi, Corfu, Ioannina, Karditsa, Evritania, Fthiotida, Chania and Rhodes). The identification of volatile components was performed using headspace solid-phase micro extraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). For the HS-SPME sampling two SPME fibers, coated with divinyl benzene/ carboxen/ polydimethy lsiloxane (DVB/ CAR/ PDMS 50/30µm) and carboxen / polydimethy lsiloxane (CAR/ PDMS 75µm) were used. Between the two SPME fibers evaluated, the DVB/ CAR/ PDMS 50/ 30 µm fiber was shown to be the best choice for all the samples. Total phenols were determined using the Folin-Ciocalteu method. Antioxidant capacity was determined by DPPH radical scavenging assay. The results indicated that the infusions and methanol extracts of chamomile flower from Fthiotida and Rodopi, respectively, showed the highest content of total phenols and the highest antioxidant capacity. On the contrary, both infusions and methanol extracts of chamomile flowers from Rhodes showed the lowest total phenols content as well as the lowest antioxidant capacity. Moreover, chamomile infusions exhibited higher total phenols content and antioxidant capacity compared to the respective methanol extracts.**

***Keywords*** **– Antioxidant Capacity, Chamomile, GC-MS, HS-SPME, Total Phenols**

## I. Introduction

Chamomile is a well-known medicinal plant species from the Asteraceae family [1]. Nowadays, chamomile is a highly favoured and much-used medicinal plant in folk and traditional medicine throughout the world and can therefore be considered as an important medicinal species [2], [3]. It is an annual herb, growing up to 20-60 cm tall with branching stems. The leaves of this herb are very fine and linear. It has a typical compositae flower, with white rays and yellow conical center that is hollow [4]. Although there are numerous varieties of chamomile, the two most popular are Roman chamomile (Chamaemelum nobile) and German chamomile (Matricaria chamomilla L. or Chamomilla recutita), both belonging to the Compositae family. Matricaria chamomilla is the only variety of this family that thrives in Greece [5]. The name "chamomile" comes from two Greek words meaning “ground apple” for its apple-like smell [6]. Chamomile prefers a sunny position in well-drained soil with a fair amount of organic matter. The best way to grow chamomile is to sow it from seed in late spring. The growing season of chamomile is relatively short-only two months. The flowers are ready to harvest when they are in full bloom [7].

Chamomile is used both internally and externally to treat an extensive list of conditions. It is used externally for wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica and rheumatic pain. The German Commission E has approved chamomile for external use for inflammation of the skin, bacterial skin diseases including those of the oral cavity and gums, and respiratory tract inflammation. Chamomile is also extensively consumed as a tea or tonic. It is used internally to treat anxiety, hysteria, nightmares, insomnia and other sleep problems, convulsions and even delirium tremens [6], [8].

In the present study the volatile constituents of chamomile (dried chamomile flower heads and chamomile infusions) from eight areas of Greece (Rodopi, Corfu, Ioannina, Karditsa, Evritania, Fthiotida, Chania and Rhodes) have been characterized using head space solid-phase micro extraction (two SPME fibers DVB/CAR/PDMS 50/30 µm and CAR/PDMS 75 µm) in conjunction with gas chromatography-mass spectrometry (GC-MS). The determination of total phenolic compounds of the infusions and methanol extracts was performed using the Folin– Ciocalteu method with gallic acid as the standard. In order to determine the antioxidant capacity of chamomile the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was applied. All determinations were performed in triplicate.

## II. Materials And Methods

### *A. Plant material*

Chamomile flowers were harvested from the previously mentioned eight different areas of Greece (Chania, Corfu, Evritania, Fthiotida, Ioannina, Karditsa, Rhodes and Rodopi). The samples were collected in May of 2010.

### *B. HS-SPME conditions*

SPME holders and coated fibres were purchased from Supelco(Bellefonte, PA, USA). For the HS-SPME sampling two SPME devices, coated with divinyl benzene /carboxen/polydimethylsiloxane(DVB/CAR/PDMS50/30µ

m) and carboxen/polydimethylsiloxane(CAR/PDMS75 μm) were used.

*B.1. Chamomile flower heads.*

0.20 g of dried chamomile flower heads were placed in 20 ml vials with 0.5 ml of saturated sodium chloride solution. The vials were sealed with septum and equilibrated for 20 min in a thermostatic bath at 80°C under stir. After the equilibration time, the SPME device was inserted into the vial by penetrating the septum and the fiber was exposed to the headspace for 30 min at 80°C to adsorb the volatiles to the fiber. After adsorption, the SPME fibre was removed from the sample and immediately inserted into the GC/MS injection port for thermal desorption (at 250ºC for 10 min) and analysis. A split injection with a ratio 1:20 was used. Before use each SPME fibre was conditioned according to the instructions of the manufacturer and was reconditioned for 15 min before sampling. Each analysis was carried out in triplicate.

*B.2. Chamomile infusions.*

To prepare the chamomile infusions, 1.0 g of dried chamomile flower heads were infused in 100 ml of distilled water at 100°C for 5 minutes and filtered through Whatman No.1 paper. 3 ml from each of the chamomile infusions were placed in 20 ml vials. Samples were further heated under the same conditions as for flower heads.

*B.3. Gas chromatography/mass spectrometry analysis.*

The analysis was carried out with a Hewlett Packard HP-6890 GC (Wilmington DE, USA) linked with a Hewlett Packard HP-5973 MS. Helium was the carrier gas at a flow rate of 1.0 ml/min. The capillary column was a HP5-MS, 30 m × 320 μm I.D. × 0.25 μm film thickness. The injector temperature was 250 °C. The ionization voltage of the MS was 70 eV and the mass range was 30-350 m/z.

*Column temperature program for chamomile flower heads.*

The initial temperature of the column was 40°C hold for 4 min, raised to 70 °C at a rate 4 °C/min, hold for 3 min, raised to 100 °C at a rate of 3 °C/min, hold for 1 min, raised to 135 °C at a rate of 3 °C/min, hold for 3 min, raised to 170 °C at a rate of 3 °C/min, hold for 1 min and finally raised to 230 °C at 5 °C/min. This temperature was maintained for 5 min.

*Column temperature program for chamomile infusions.*

The initial temperature of the column was 40 °C hold for 3 min, raised to 160 °C at a rate 18 °C/ min, hold for 4 min, raised to 170 °C at a rate 2 °C/min, hold for 3 min, raised to 180 °C at a rate of 5 °C/min, hold for 3 min and finally raised to 230 °C at 5 °C/min. This temperature was maintained for 4 min.

*B.4. Identification of volatiles.*

The volatile compounds were identified by comparison of their retention indices (RIs) with those found in the literature and by comparison of the mass spectra of each compound with the Wiley 275 L mass spectral library as well as with the NIST mass spectral library.

*B.5. Total Phenols.*

*B.5.1. Sample preparation.*

Chamomile infusion: 1.5 g of dried chamomile flower heads were infused in 100 ml of distilled water at 100°C for five minutes and filtered through Whatman No.1 paper.

Chamomile methanol extract: 0.40 g of dried chamomile flower heads were sonicated with 20 ml of methanol in an ultra sonicator bath (Elma Ultrasonic, Germany) at a controlled temperature (30 ± 0.5 °C) for sixty minutes. After extraction, the extract was filtered through Whatman No.1 paper. All filtrations were carried out in triplicate.

*B.5.2. Folin - Ciocalteu method.*

Total phenol content in chamomile infusions and methanol extracts was determined spectrophotometrically by the Folin–Ciocalteu method, according to Singleton and Rossi (1965) [9]. The method is based on the reduction of phosphotungstic acid ($H_3P[W_3O_{10}]_4$) in alkaline solution to phosphotungstic blue. The absorbance of formed phosphotungstic blue is proportional to the number of aromatic phenolic groups and is used for their quantification, with gallic acid as the standard. Briefly, the extract (0.2 ml) was mixed with 0.5 ml of Folin – Ciocalteu reagent. After 1 min, 1.5 ml of 7.5% sodium carbonate solution and 7.8 ml distilled water were added and mixed well. The samples were left to stand for 2 h at room temperature and the absorbance at 760 nm was measured on a UV–visible spectrophotometer. The results were expressed in milligram gallic acid equivalents (GAE) per liter. All determinations were performed in triplicate.

*B.6. Antioxidant Capacity.*

The antioxidant capacity of chamomile was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This method is based on the reduction of stable DPPH radical by antioxidants. In the presence of antioxidants the purple color of the DPPH radical solution changes to a bright yellow and the intensity of this change can be monitored spectrophotometrically. The samples were analyzed according to the method reported by Horzic et al. (2009) [10]. In brief, a volume of 3.8 ml of 0.1 mmol/l DPPH radical solution in methanol was added to 0.2 ml of sample and the free radical scavenging capacity of the sample was evaluated by measuring the absorbance at 517 nm immediately after the addition of DPPH* (t = 0) and at 1 min intervals, until the radical scavenging reaction reached to steady state (after 45 min). The results were expressed as the percentage of reduction (inhibition) of the DPPH* (Q), which is defined by following expression: $Q = [(A_0 - A_S) / A_0] \times 100$ where $A_0$ is the initial absorbance and $A_S$ is the value of absorbance after the reaction reached to steady state. All determinations were performed in triplicate.

*B.7 Growing Degree Days Calculation.*

Climatic data during the experimental period, from January to May of 2010, were collected from the nearest weather stations of Hellenic National Meteorological Service. Then, accumulated growing degree days (GDD) were calculated for each month using the mean-minus-base method [11], [12], [13] using the formula:

GDD = [(maximum daily temperature + minimum daily temperature)/2] – base temperature.

The basal growth temperature was set at 10°C as it reflects the essential temperature for the initiate development of chamomile [14]. Also, a lower limit of 10°C was set for the minimum temperature. Growing degree days were summed from 1 January of 2010 according to Hendrickson et al. [15].

*B.8. Statistical analysis.*

An analysis of variance was used to test differences in chamomile methanol extract and infusion total phenols across the different areas. For statistically different parameters ($P < 0.05$), means were separated using the Least Significant Difference (LSD) comparison test. Correlation analyses were also carried out to examine relationships between climatic variables (Growing Degree Days and sunshine duration) and total phenols. Climatic data were obtained from Hellenic National Meteorological Service concerning the period from March through May of 2010. Data analyses were done using SPSS statistical software package.

## III. Results and Discussion.

*1. Identification of volatiles.*

A typical gas chromatogram of the chamomile flower heads from the district of Ioannina using the DVB/CAR/PDMS 50/30μm fiber and the HP5-MS column is shown in Figure 1. The respective chromatogram of the chamomile flower heads from the same region using the CAR/PDMS 75μm fiber and the HP5-MS column is shown in Figure 2.

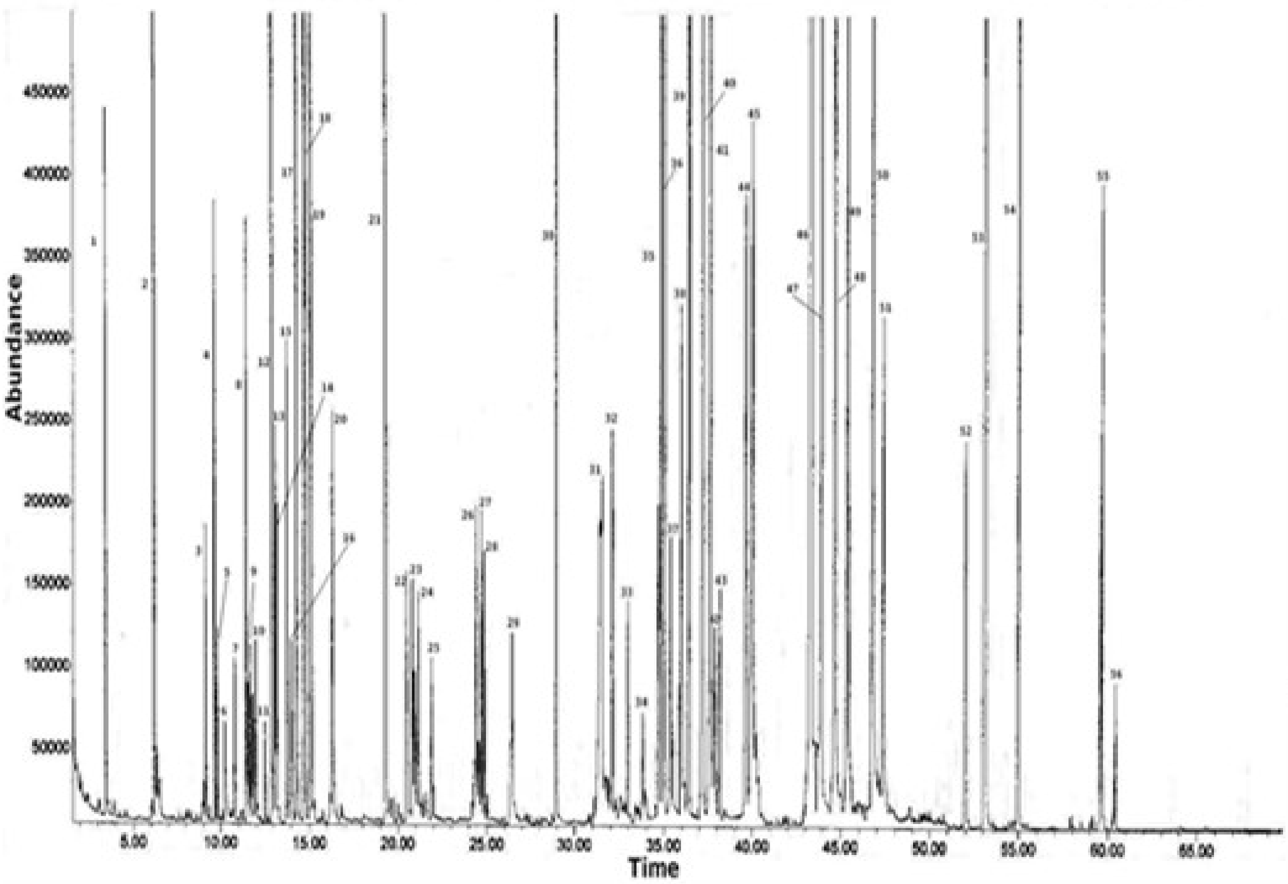


Fig.1. Gas chromatogram of the chamomile flower heads from Ioannina district using the DVB/CAR/PDMS 50/30 μm fiber and the HP5-MS column. (1) internal standard, (2) butanoic acid,2-methyl-ethyl ester, (3) α-pinene, (4) camphene, (5) propyl 2-methylbutyrate, (6) benzaldehyde, (7) L-β-pinene, (8) 6-methyl-5-hepten-2-one, (9) myrcene, (10) yomogi alcohol, (11) α-terpinene, (12) p-cymene, (13) limonene, (14) 1,8-cineole, (15) cis-ocimene, (16) butyl 2-methyl butanoate, (17) trans-β-ocimene, (18) γ-terpinene, (19) artemisia ketone, (20) artemisia alcohol, (21) camphor, (22) borneol L, (23) (-)-lavandulol, (24) terpinen-4-ol, (25) α-terpineol, (26) cis-3-hexenyl-isovalerate, (27) hexyl isovalerate, (28) trans-2-hexenyl isovalerate, (29) 4,8-dimethyl-nona-3,8-dien-2-one (30) decanoic acid methyl ester, (31) decanoic acid, (32) benzyl valerate, (33) β-caryophyllene, (34) (+)-Aromadendrene, (35) alloaromadendrene, (36) trans-β-farnesene, (37) germacrene-D, (38) γ-curcumene, (39) germacrene-B, (40) γ-cadinene, (41) d-cadinene, (42) dodecanoic acid methyl ester, (43) α-cadinene, (44) nerolidol, (45) spathulenol, (46) α-cadinol, (47) T-cadinol, (48) curvulol, (49) α-bisabolol, (50) 7-methoxy-coumarin, (51) tetradecanoic acid methyl ester, (52) 2-pentadecanone,6,10,14-trimethyl, (53) en-in-dicycloether, (54) hexadecanic acid methyl ester, (55) 9,12,15-octadecatrienoic acid methyl ester, (56) linoleic acid.

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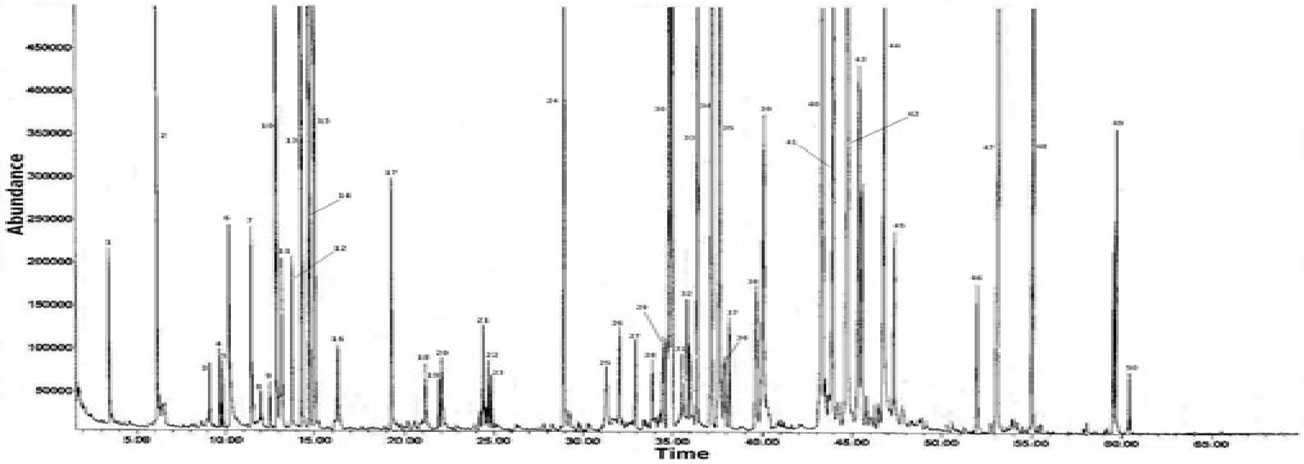


Fig.2. Gas chromatogram of the chamomile flower heads from Ioannina district using the CAR/PDMS 75 µm fiber and the HP5-MS column. (1) internal standard, (2) butanoic acid,2-methyl-ethyl ester, (3) α-pinene, (4) camphene, (5) propyl 2-methylbutyrate, (6) benzaldehyde, (7) 6-methyl-5-hepten-2-one, (8) myrcene, (9) α-terpinene, (10) p-cymene, (11) limonene, (12) cis-ocimene, (13) trans-β-ocimene, (14) γ-terpinene, (15) artemisia ketone, (16) artemisia alcohol, (17) camphor, (18) terpinen-4-ol, (19) α-terpineol, (20) 2-methoxy-4-methylphenol, (21) cis-3-hexenyl-isovalerate, (22) hexyl isovalerate, (23) trans-2-hexenyl isovalerate, (24) decanoic acid methyl ester, (25) decanoic acid, (26) benzyl valerate, (27) β-caryophyllene, (28) (+)-Aromadendrene, (29) alloaromadendrene, (30) trans-β-farnesene, (31) germacrene-D, (32) γ-curcumene, (33) germacrene-B, (34) γ-cadinene, (35) d-cadinene, (36) dodecanoic acid methyl ester, (37) α-cadinene, (38) nerolidol, (39) spathulenol, (40) α-cadinol, (41) T-cadinol, (42) curvulol, (43) α-bisabolol, (44) 7-methoxy-coumarin, (45) tetradecanoic acid methyl ester, (46) 2-pentadecanone,6,10,14-trimethyl, (47) en-in-dicycloether, (48) hexadecanic acid methyl ester, (49) 9,12,15-octadecatrienoic acid methyl ester, (50) linoleic acid.

Table 1 shows the volatile compounds of chamomile flower heads from the different areas of Greece that were identified using two SPME fibers and the HP5-MS column. In the investigated samples, a total of 69 volatile substances were identified belonging to the general classes of terpenes (52 compounds), esters (9 compounds), ketones (2 compounds), acids (2 compounds), 1 aldehyde, 1 phenol, 1 coumarin and 1 dicycloether.

Table 1 Identification data and the volatile constituents of chamomile flower heads from different areas of Greece, using two SPME fibers and HP5-MS column. 1: Rodopi, 2: Ioannina, 3: Corfu, 4: Fthiotida, 5: Rhodes, 6: Chania, 7: Evritania, 8: Karditsa

A: DVB/CAR/PDMS 50/30µm, B: CAR/PDMS 75µm.

| **District** | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPME Fibers** | | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* |
| **RI** | **Volatile Constituents** | | | | | | | | | | | | | | | | |
| 933 | **α-pinene** | √ | - | √ | √ | √ | √ | - | - | √ | - | √ | √ | - | - | √ | - |
| 947 | **camphene** | - | - | √ | √ | √ | √ | - | - | √ | - | √ | √ | √ | - | - | - |
| 975 | **L-β- pinene** | - | - | √ | - | √ | - | - | - | - | - | √ | √ | - | - | - | - |
| 991 | **6-methyl-5-hepten-2-one** | - | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | √ | - | - |
| 994 | **myrcene** | - | - | √ | √ | - | - | - | - | - | - | - | - | - | - | - | - |
| 1003 | **yomogi alcohol** | - | - | √ | - | √ | - | - | - | - | - | √ | - | - | - | - | - |
| 1014 | **α-terpinene** | - | - | √ | √ | - | - | - | - | - | - | - | - | - | - | - | - |
| 1021 | **p-cymene** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1025 | **limonene** | √ | √ | √ | √ | √ | √ | √ | √ | - | - | √ | √ | √ | √ | - | - |
| 1027 | **1,8-cineole** | - | - | √ | - | √ | √ | √ | √ | √ | √ | - | - | - | - | √ | √ |
| 1038 | **cis-ocimene** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1064 | **artemisia ketone** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | √ | √ |
| 1087 | **artemisia alcohol** | - | - | √ | √ | √ | - | - | - | √ | √ | √ | √ | - | - | - | - |
| 1146 | **camphor** | - | - | √ | √ | √ | √ | √ | - | √ | √ | √ | √ | √ | - | - | - |
| 1175 | **(-)-lavandulol** | - | - | √ | - | - | - | √ | - | √ | - | √ | - | - | - | - | - |
| 1182 | **terpinen-4-ol** | - | - | √ | √ | √ | √ | - | - | - | - | - | - | - | - | - | - |
| 1196 | **α-terpineol** | - | - | √ | √ | √ | √ | √ | - | √ | - | - | - | - | - | √ | - |
| 1245 | **cis-3-hexenyl isovalerate** | - | - | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | - | - | - |
| 1251 | **hexyl isovalerate** | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | - | - | - | - |
| 1254 | **trans-2-hexenyl isovalerate** | - | - | √ | √ | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - |
| 1395 | **benzyl valerate** | - | - | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | - | - | - |
| 1415 | **β-caryophyllene** | √ | √ | √ | √ | √ | - | √ | √ | - | - | √ | √ | - | - | √ | √ |
| 1435 | **(+)-aromadendrene** | √ | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | √ | √ |

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| District | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SPME Fibers | | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* |
| 1461 | trans-β-farnesene | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1478 | germacrene-D | √ | √ | √ | √ | √ | - | √ | √ | √ | - | √ | √ | √ | - | √ | √ |
| 1048 | trans-β-ocimene | √ | √ | √ | √ | √ | √ | √ | - | √ | √ | √ | √ | - | - | √ | √ |
| 1058 | γ-terpinene | √ | - | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | - | √ | √ |
| 1480 | γ-curcumene | - | - | √ | √ | - | √ | √ | √ | √ | - | √ | √ | - | - | √ | √ |
| 1483 | AR-curcumene | - | - | - | - | - | √ | - | - | - | - | - | - | - | √ | - | - |
| 1731 | tetradecanoic acid methyl ester | - | - | √ | √ | √ | √ | - | - | √ | √ | - | - | - | - | - | - |
| 1493 | germacrene-B | √ | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | √ | - | √ | √ |
| 1520 | d-cadinene | - | - | √ | √ | √ | √ | √ | √ | √ | - | √ | √ | - | - | √ | √ |
| 1531 | α-cadinene | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1562 | nerolidol | - | - | √ | √ | √ | √ | - | - | - | - | - | - | - | - | - | - |
| 1570 | spathulenol | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1641 | α-cadinol | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | - | - | - | - |
| 1653 | T-cadinol | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1684 | α-bisabolol | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | √ | √ |
| 1879 | en-in-dicycloether | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 2109 | linoleic acid | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1391 | β-elemene | √ | √ | - | - | - | - | - | √ | √ | √ | √ | √ | √ | - | - | - |
| 1482 | b-selinene | √ | √ | - | - | - | - | - | - | √ | √ | √ | √ | - | - | - | - |
| 1654 | bisabolol oxide B | √ | √ | - | - | - | - | - | - | √ | √ | √ | √ | √ | √ | √ | √ |
| 1688 | bisabolone oxide | √ | √ | - | - | - | - | - | - | √ | √ | √ | √ | √ | √ | √ | √ |
| 1509 | E,E-alpha-farnesene | √ | √ | - | - | - | - | - | - | √ | √ | √ | - | - | - | √ | √ |
| 1508 | b-bisabolene | √ | √ | - | - | - | - | - | - | - | - | - | - | √ | - | √ | √ |
| 1282 | 4,8-dimethyl-nona-3,8-dien-2-one | - | - | √ | - | √ | - | - | - | √ | √ | √ | - | - | - | - | - |
| 1716 | 7-methoxy-coumarin | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1723 | azulene 7-ethyl-1,4-dimethyl | - | - | - | - | - | - | - | - | √ | √ | - | √ | - | - | - | - |
| 1378 | a-copaene | - | - | - | - | - | - | - | - | √ | - | √ | √ | - | - | √ | √ |
| 1510 | γ-cadinene | - | - | √ | √ | √ | √ | √ | √ | - | - | - | √ | - | - | - | - |
| 1746 | bisabolol oxide A | √ | √ | - | - | - | - | √ | - | √ | √ | √ | √ | √ | √ | √ | √ |
| 1670 | curvulol | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | - | - | √ | √ |
| 853 | butanoic acid, 2-methyl-,ethyl ester | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | - | - | √ | √ |
| 950 | propyl,2-methylbutyrate | - | - | √ | √ | √ | √ | √ | √ | - | √ | √ | - | - | - | - | - |
| 1043 | butyl 2-methylbutanoate | - | - | √ | - | - | √ | √ | √ | - | - | - | - | - | - | - | - |
| 960 | benzaldehyde | - | √ | √ | √ | - | √ | √ | √ | - | √ | √ | √ | - | √ | - | √ |
| 973 | sabinene | √ | - | - | - | - | - | - | - | - | - | - | - | - | - | √ | - |
| 1169 | borneol L | √ | - | √ | - | - | - | - | - | - | - | √ | √ | √ | √ | - | - |
| 1334 | decanoic acid methyl ester | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1382 | decanoic acid | √ | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - | - |
| 1525 | dodecanoic acid methyl ester | - | - | √ | √ | - | - | - | - | √ | - | - | - | - | - | - | - |
| 1200 | 2-methoxy-4-methylphenol | - | - | - | √ | - | - | - | √ | - | √ | - | - | - | - | - | - |
| 1848 | 2-pentadecanone,6,10,14-trimethyl | - | - | √ | √ | √ | √ | - | - | √ | √ | - | - | - | - | - | - |
| 1485 | valencene | √ | √ | - | - | - | - | - | - | √ | - | - | - | √ | - | - | - |
| 1311 | carvacrol | √ | - | - | - | - | - | - | - | - | - | - | - | √ | √ | √ | √ |
| 1935 | hexadecanoic acid methyl ester | √ | √ | √ | √ | √ | √ | √ | √ | - | √ | √ | √ | √ | √ | √ | √ |
| 1457 | alloaromadendrene | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | - | - | √ | √ |
| 2085 | 9,12,15-octadecatrienoic acid methyl ester | - | - | √ | √ | √ | - | √ | √ | - | - | - | - | - | - | √ | √ |

Fifty-five compounds were detected in dried chamomile flower heads from Ioannina using DVB/CAR/PDMS 50/30 μm fiber, whereas fewer (forty-nine) compounds were detected in dried chamomile flower heads from the same region using CAR/PDMS 75μm fiber. The same trend was also observed for the samples from the other regions. Therefore, the DVB/CAR/PDMS 50/30μm fiber was shown to be the best choice for all the samples.

As reported previously, chamomile has a warm, sweet, herbaceous scent that is relaxing and calming and the compounds responsible for chamomile distinctive aroma are mainly trans-β-farnesene, b-bisabolene, germacrene-B, germacrene-D, α-bisabolol, spathulenol, bisabolol oxide A and bisabolol oxide B [16].

Figures 3 and 4 shows the typical gas chromatograms of the chamomile infusion from the district of Ioannina using the DVB/CAR/PDMS 50/30 μm fiber and the CAR/PDMS 75μm fiber, respectively.

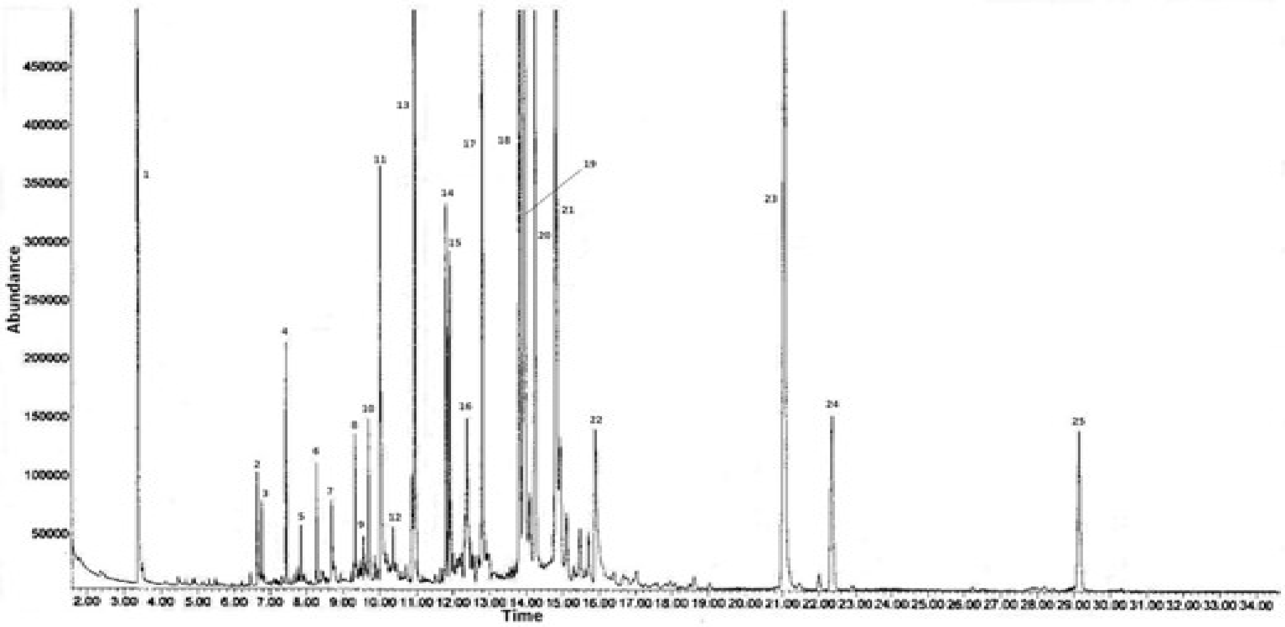

Fig.3. Gas chromatogram of the chamomile infusion from Ioannina district using the DVB/CAR/PDMS 50/30 μm fiber and the HP5-MS column. (1) internal standard, (2) 6-methyl-5-hepten-2-one, (3) decane, (4) artemisia ketone, (5) nonanal, (6) camphor, (7) decanal, (8) 4,8-dimethyl-nona-3,8-dien-2-one, (9) carvacrol, (10) decanoic acid methyl ester, (11) decanoic acid, (12) benzyl valerate, (13) trans-β-farnesene, (14) γ-cadinene, (15) d-cadinene, (16) nerolidol, (17) spathulenol, (18) gossonorol, (19) α-cadinol, (20) T-cadinol, (21) α-bisabolol, (22) 7-methoxy-coumarin, (23) en-in-dicycloether, (24) hexadecanoic acid methyl ester, (25) 9,12,15-octadecatrienoic acid methyl ester.

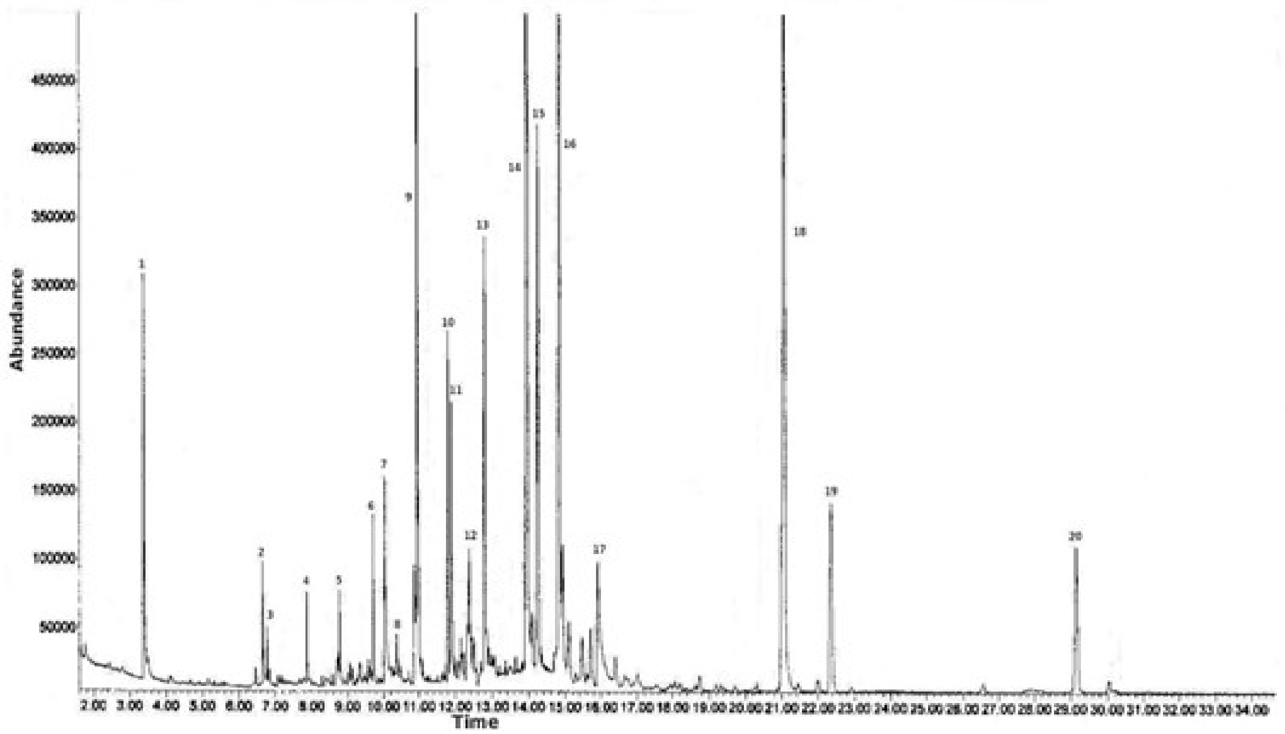

Fig.4. Gas chromatogram of the chamomile infusion from Ioannina district using the CAR/PDMS 75 μm fiber and the HP5-MS column. (1) internal standard, (2) 6-methyl-5-hepten-2-one, (3) decane, (4) nonanal, (5) decanal, (6) decanoic acid methyl ester, (7) decanoic acid, (8) benzyl valerate, (9) trans-β-farnesene, (10) γ-cadinene, (11) d-cadinene, (12) nerolidol, (13) spathulenol, (14) α-cadinol, (15) T-cadinol, (16) α-bisabolol, (17) 7-methoxy-coumarin, (18) en-in-dicycloether, (19) hexadecanoic acid methyl ester, (20) 9,12,15-octadecatrienoic acid methyl ester.

The volatile constituents of chamomile infusions are presented in Table 2. In the investigated samples, a total of 28 volatile substances were identified belonging to the general classes of terpenes (20 compounds), esters (4 compounds), 1 ketone, 1 acid, 1 coumarin and 1 dicycloether.

Table 2 Identification data and the volatile constituents of chamomile infusions from different areas of Greece, using two SPME fibers and HP5-MS column.1: Rodopi, 2: Ioannina, 3: Corfu, 4: Fthiotida, 5: Rhodes, 6: Chania, 7: Evritania, 8: Karditsa

A: DVB/CAR/PDMS 50/30μm, B: CAR/PDMS 75μm.

| **District** | | **1** | | **2** | | **3** | | **4** | | **5** | | **6** | | **7** | | **8** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPME Fibers** | | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* |
| **RI** | **Volatile Constituents** | | | | | | | | | | | | | | | | |
| 991 | **6-methyl-5-hepten-2-one** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1000 | **decane** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1024 | **artemisia ketone** | - | - | √ | - | - | - | √ | √ | √ | - | - | - | - | - | - | - |
| 1123 | **camphor** | - | - | √ | - | √ | - | - | - | - | - | - | - | - | - | - | - |
| 1106 | **nonanal** | √ | √ | √ | √ | √ | √ | √ | - | √ | √ | √ | - | √ | √ | √ | √ |
| 1208 | **decanal** | √ | √ | √ | √ | √ | √ | - | - | √ | √ | √ | - | √ | √ | √ | √ |
| 1279 | **4,8-dimethyl-nona-3,8-dien-2-one** | - | - | √ | - | √ | √ | - | - | √ | √ | - | - | - | - | - | - |
| 1307 | **carvacrol** | - | - | √ | - | √ | - | - | - | - | - | - | - | √ | √ | √ | √ |
| 1324 | **decanoic acid methyl ester** | √ | √ | √ | √ | √ | √ | √ | √ | - | - | √ | √ | √ | √ | √ | √ |
| 1363 | **decanoic acid** | √ | √ | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1402 | **benzyl valerate** | - | - | √ | √ | - | - | √ | √ | - | - | - | - | - | - | - | - |
| 1459 | **trans-β-farnesene** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1527 | **γ-cadinene** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1534 | **d-cadinene** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1595 | **spathulenol** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1566 | **nerolidol** | - | - | √ | √ | √ | √ | - | - | - | - | - | - | - | - | - | - |
| 1643 | **gossonorol** | - | - | √ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1651 | **α-cadinol** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | √ | - | - | - | - |
| 1665 | **T-cadinol** | - | - | √ | √ | √ | √ | √ | √ | - | - | - | - | - | - | - | - |
| 1692 | **α-Bisabolol** | - | - | √ | √ | - | - | - | - | - | - | - | - | - | - | - | - |
| 1733 | **7-methoxy-coumarin** | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | - | - | - | √ | - |
| 1897 | **en-in-dicycloether** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1931 | **hexadecanoic acid, methyl ester** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 2105 | **9,12,15-octadecatrienoic acid methyl ester** | - | - | √ | √ | √ | √ | √ | √ | - | - | √ | √ | √ | √ | √ | √ |
| 1745 | **azulene 7-ethyl-1,4-dimethyl** | √ | - | - | - | - | - | - | - | √ | √ | - | √ | √ | - | - | - |
| 1669 | **alpha-bisabolol oxide B** | √ | √ | - | - | - | - | - | - | √ | √ | √ | √ | √ | √ | √ | √ |
| 1702 | **bisabolone oxide** | √ | √ | - | - | - | - | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| 1761 | **bisabolol oxide A** | √ | √ | - | - | - | - | - | - | √ | √ | √ | √ | √ | √ | √ | √ |

Using DVB/CAR/PDMS 50/30μm fiber, more compounds were identified in all samples in comparison with CAR/PDMS 75μm fiber. Specifically, twenty-four compounds were identified in chamomile infusion from Ioannina district using DVB/CAR/PDMS 50/30μm fiber followed by samples from Corfu district (twenty compounds), Fthiotida district (eighteen compounds), Evritania and Karditsa districts (fourteen compounds), Rodopi, Rhodes and Chania districts (thirteen compounds).

Using CAR/PDMS 75μm fiber, nineteen compounds were identified in chamomile infusion of Ioannina district followed by samples of Corfu district (eighteen compounds), Fthiotida district (seventeen compounds), Evritania and Karditsa districts (thirteen compounds), Rodopi, Rhodes and Chania districts (twelve compounds). Therefore, as in case of chamomile flower heads, the DVB/CAR/PDMS 50/30μm fiber was shown to be the best choice for the analysis of all chamomile infusion samples.

*2. Total phenols and antioxidant capacity.*

Analysis of variance showed that total phenols content both in chamomile infusion and methanol extract varied significantly ($P<0.05$) with sample origin (Table 3). The results indicate that among the chamomile infusions, the lowest total phenols content (expressed as mg gallic acid equivalents/l) was found in chamomile flowers from Rhodes (25.12 mg/l GAE), while the highest was found in chamomile flowers from Fthiotida (41.67 mg/l GAE). The order of total phenols content values was: Fthiotida > Rodopi > Corfu > Ioannina > Karditsa > Chania > Evritania > Rhodes. Totals phenols content in chamomile infusions and methanol extracts negatively ($P<0.05$) correlated with Growing Degree Days ($r=-0.793$ and $r=-0.420$, respectively) and sunshine duration ($r=-0.586$ and $r=-0.333$, respectively). It seems that differences in climatic environment results in differences in total phenols content.

Table 3 Contents of total phenols in chamomile infusions and methanol extracts. Values are expressed as means in mg/l ± SD (n=3).

| Sample origin | Chamomile infusion | Chamomile methanol extract |
|---|---|---|
| Chania | 31.89 ± 0.03 a | 28.85 ± 0.04 a |
| Corfu | 39.70 ± 0.04 b | 22.48 ± 0.04 b |
| Evritania | 30.50 ± 0.04 c | 22.12 ± 0.04 c |
| Fthiotida | 41.67 ± 0.02 d | 25.30 ± 0.03 d |
| Ioannina | 39.12 ± 0.03 e | 23.91 ± 0.03 e |
| Karditsa | 38.44 ± 0.03 f | 29.07 ± 0.04 f |
| Rhodes | 25.12 ± 0.03 g | 20.73 ± 0.03 g |
| Rodopi | 40.33 ± 0.02 h | 30.37 ± 0.03 h |

Means followed by the same letter do not significantly differ from each other.

Also, higher total phenols content is observed in the methanol extracts of chamomile flowers from Rodopi (30.37 mg/l GAE) and the lowest one in chamomile from Rhodes (20.73 mg/l GAE). The order of total phenols content values in the methanol extracts of chamomile flowers was: Rodopi > Karditsa > Chania > Fthiotida > Ioannina > Corfu > Evritania > Rhodes. Moreover, both infusions and methanol extracts of chamomile flowers from Rhodes showed the lowest total phenols content. Furthermore, the amounts of total phenols in the chamomile infusions were higher than the respective methanol extracts.

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH*) radical scavenging assay is one of the most extensively used antioxidant assays for plant materials. When DPPH* reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The change in color (from purple to yellow) was measured at 517 nm. So, the ability of chamomile infusions and methanol extracts to scavenge free radical DPPH* is proportional to the antioxidant capacity. The results of antioxidant capacity of chamomile infusions and methanol extracts evaluated by DPPH* assay are presented in Table 4 and have been expressed as the percentage of inhibition of the DPPH*. Antioxidant capacity both of chamomile infusions and methanol extracts is strongly ($P<0.01$) affected by the sample origin.

Table 4 Antioxidant capacity of chamomile infusions and methanol extracts evaluated by DPPH* assay. Values are expressed as % of inhibition ± SD (n = 3).

| Sample origin | Chamomile infusion | Chamomile methanol extract |
|---|---|---|
| Chania | 84.64 ± 0.03 a | 78.64 ± 0.13 a |
| Corfu | 90.09 ± 0.16 b | 57.73 ± 0.69 b |
| Evritania | 81.84 ± 0.05 c | 53.93 ± 0.35 c |
| Fthiotida | 92.04 ± 0.18 d | 68.24 ± 0.57 d |
| Ioannina | 86.84 ± 0.17 e | 59.39 ± 0.71 e |
| Karditsa | 85.85 ± 0.43 f | 79.94 ± 0.09 f |
| Rhodes | 80.86 ± 0.42 g | 31.29 ± 0.25 g |
| Rodopi | 90.75 ± 0.09 b | 80.92 ± 0.21 h |

Means followed by the same letter do not significantly differ from each other.

As shown in Table 4, among the chamomile extracts, the ability to scavenge the free radical DPPH is very high in chamomile infusions. The highest and lowest antioxidant capacity was observed in the infusions of chamomile flowers from Fthiotida (92.04%) and Rhodes (80.86%), respectively. Moreover, both infusions and methanol extracts of chamomile flowers from Rhodes showed the lowest antioxidant capacity. The antioxidant capacity of the chamomile infusions decreased in the following order: Fthiotida > Rodopi > Corfu > Ioannina > Karditsa > Chania > Evritania > Rhodes, which is the same as the order of total phenols content. Likewise, the order of antioxidant capacity of chamomile methanol extracts is also the same as the order of total phenols content.

The antioxidant capacity of infusions and methanol extracts were strongly correlated with Growing Degree Days ($r=-0.594$; $P<0.01$ and $r=-0.553$; $P<0.01$, respectively) and sunshine duration ($r=-0.410$; $P<0.05$ and $r=-0.387$; $P<0.05$, respectively). The results indicated that the amount of phenolics present in the infusions and methanol extracts was the key determinant for their antioxidant capacities, expressed as the ability to scavenge free radical DPPH.

## IV. Conclusion

A great number of volatile/aroma compounds were detected in dried chamomile flower heads, most of them belonging to the general class of terpenes.

The antioxidant capacity of chamomile infusions was directly related to their total phenol content and was strongly affected by the sample origin.

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