



Total Antioxidant Capacity and Phenolic Content of 17 Mediterranean Functional Herbs and Wild Green Extracts from North Aegean, Greece [†]

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[†] Presented at the 2nd International Electronic Conference on Foods; Future Foods and Food Technologies Science for a Sustainable World; 15–30 October 2021; Available online: <https://foods2021.sciforum.net/>.

Abstract: Functional foods are considered beneficial to human health due to their high nutritional values. Currently, there is development in research to identify natural functional food sources high in bioactive compounds, such as antioxidants and polyphenols, as they are shown to contribute towards decreasing oxidative stress and maintaining well-being in human health. The objective of the present study was to determine the total antioxidant capacity and total phenolic content of Mediterranean medicinal natural herbs and wild greens, aiming to create a database of medicinal plants for future potential use as functional food ingredients. A total of seventeen selected herbs and wild greens from Lemnos Island (Greece) were purchased between June and July of 2021. Extracts were prepared with an ultrasound water bath at 70 °C for 60 min. The antioxidant capacity was measured with ferric reducing antioxidant power (FRAP) assay and the total polyphenolic content was determined with the Folin–Ciocalteu reagent method. The mean phenolic values ranged from 0.18 to 2282.80 mg of gallic acid/g of sample, whereas *Hypericum perforatum* L. and *Hypericum perforatum* L., *Salvia* spp., and *Sideritis* sp. displayed the highest contents ($p < 0.05$). *Melissa officinalis* L., *Mentha Spicata*, and *Thymbra capitata* L. obtained the highest total antioxidant capacity values greater than 12.20 mmol/L, with an average range of 0.06 to 30.58 mmol of Fe^{2+} /L of extract ($p < 0.05$). *Pistacia lentiscus* var. *chia*, *Crataegus azarolus* L., and *Pancreatium Maritimum* obtained the lowest contents from both methods. Studies have shown that medicinal plants with total antioxidant values higher than 0.87 mmol/L could be applicable in the fortification of food products (i.e., yogurt). Therefore, this study suggests that most of the above medicinal plants could be a potential source of antioxidants for functional food applications while their use can enhance Mediterranean diet principles.

Keywords: natural health products; sustainable functional foods; herbs; wild greens; bioactive compounds; FRAP assay; Folin–Ciocalteu assay



Citation: Kaloteraki, C.; Almpounioti, K.; Potsaki, P.; Bousdouni, P.; Kandyliari, A.; Koutelidakis, A.E. Total Antioxidant Capacity and Phenolic Content of 17 Mediterranean Functional Herbs and Wild Green Extracts from North Aegean, Greece. *Biol. Life Sci. Forum* **2021**, *6*, 43. <https://doi.org/10.3390/Foods2021-11003>

Academic Editor: Antonello Santini

Published: 14 October 2021

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1. Introduction

In the past decades, there has been an estimated growing population of over eight billion people around the globe, and more than three million face many chronic diseases, such as cardiovascular disease and diabetes [1]. Balanced diets and healthy food choices can play a crucial role in the prevention of health-related diseases. Therefore, there is a high demand globally and in local markets to explore innovative food technologies and to identify food sources that can contribute to healthy and sustainable living [2,3].

Currently, the research community focuses on investigating foods that contain bioactive compounds. These foods rich in bioactive compounds are called functional foods. The term was first used in Japan around the 1980s and has been defined as processed or natural

foods that can have positive health outcomes due to their nutritional value from their bioactive compounds [4–6]. Foods are characterized as functional due to their content of bioactive compounds including antioxidants, polyphenols, carotenoids, fiber, phytosterols, etc. [7]. Beneficial sources of foods, with a significant content of antioxidants and especially polyphenols, have been detected from fruits, vegetables, medicinal plants, wild greens and others [8,9].

Epidemiological studies have shown that functional food consumption is a critical factor with possible physiological health benefits towards many chronic diseases, such as cardiovascular diseases, diabetes, metabolic syndrome and immune response [10–12]. Antioxidant compounds have been identified to play a vital role in decreasing oxidative stress and maintaining well-being in human health. Indicatively, the consumption of medicinal plants has been associated with the prevention of metabolic disorders and diabetes, and these plants possess anti-cancer, hepatoprotective, and anti-depressive properties [13].

The aim of the present study was to determine the total antioxidant capacity and total phenolic content of seventeen Mediterranean medicinal natural herbs and wild greens. All the herbs and wild greens were harvested and/or purchased from local suppliers from Lemnos Island, Greece. In the past several years, many researchers have studied the beneficial properties of herbs and wild edible greens towards human health in the Mediterranean diet [14].

This study aims to create a database of medicinal plants for future potential use as functional food ingredients [15]. The present research provides valuable information regarding the value of local products from the North Aegean and especially Lemnos Island and their use as natural food additives. All the information obtained from this study could be applicable for future potential use of these products as functional food ingredients [16,17].

2. Materials and Methods

Herbs and wild greens originating from the Islands of North Aegean were purchased/collected at the time of their maximum yield from the local market of Lemnos Island, Greece, between June and July of 2021. Samples of the same herb/wild green were pooled together and dried in a shady place with low humidity. Then, they were kept in a dry place in sealed bags until extraction. Extraction of samples was conducted in distilled water. More specifically, the samples were the following: *Ocimum basilicum* L. (basil), *Crataegus azarolus* L. (crataegus), *Salvia* spp. (mountain tea), *Sideritis* sp. (sage), *Thymus* sp. (thymus), *Thymbra capitata* L. (thymbra), *Origanum* sp. (oregano), *Pistacia lentiscus* var. *chia* (mastiha), *Melissa Officinalis* L. (lemon balm), *Mentha Spicata* (mint), *Matricaria chamomilla* L. (chamomile), *Rosmarinus officinalis* L. (rosemary), *Hypericum perforatum* L. and *Hypericum perforatum* L. (St John's-wort), and *Otanthus maritimes* (cotton weed), and wild greens *Crithmum maritimum* L. (sea fennel), *Salicornia europaea* L. (common glasswort), and *Pancratium maritimum* (sea daffodil). Extracts were prepared by adding 2 g of herbs/wild greens to 100 mL of dH₂O in the ultrasound (Elmasonic P, Elma) for 60 min at 70 °C at 80 Hz. Filtration of the extracts was performed by filter paper. After the extracts were cold, 1:5 to 1:20 dilutions were prepared for analysis. Each extraction was prepared in triplicate and then further analyzed for its total phenolic and antioxidant content. The graphical scheme of the study design is presented in Figure 1.

Total phenolic content was determined with Folin–Ciocalteu assay. Folin–Ciocalteu reagent (20 µL) was added along with the dilution of the plant extract (50 µL) and Na₂CO₃ (20 µL) in a 96-well plate and remained 30 min in dark environment. The absorbance of the solution was measured at 765 nm with a spectrophotometer (Tecan Spark) according to the method described by Spanos et al. [18]. Analysis was carried out in triplicate. A standard curve with known concentration of gallic acid (GA) was performed for the quantification of the measurements. Results are expressed as GA equivalents per gram of sample.

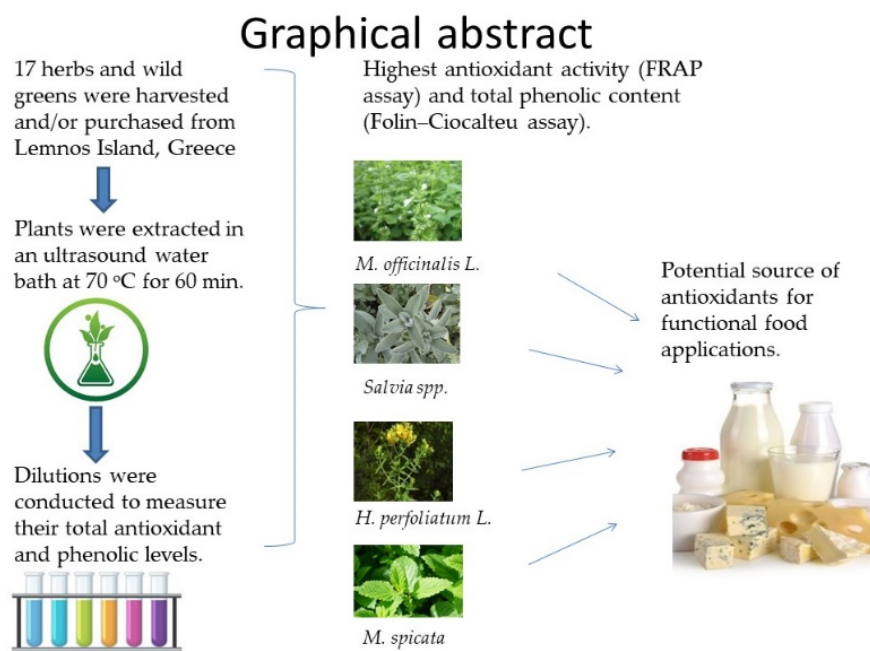


Figure 1. Graphical scheme of the study design.

Total antioxidant capacity was determined with a FRAP assay. FRAP assay (80 μ L) and dilution of plant extract (20 μ L) were added in 96-well plate for 30 min in dark environment. The absorbance of the solution measured at 595 nm according to Cao et al. and Kapsokefalou et al. [19,20]. A standard curve with known concentration of Fe^{2+} (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was performed for the quantification of the measurements. Analysis was carried out in triplicate. Results are expressed as Fe^{2+} mmol/L.

Statistical analysis was performed using the SPSS package, version 16.1 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean $\pm$ standard deviation (SD). The non-parametric Kruskal–Wallis test was used to statistically compare values between the different food groups due to the small sample size (9 replicates in total per sample).

3. Results and Discussion

There is considerable knowledge regarding the medicinal properties of edible herbs and wild greens from the North Aegean Islands of Greece. Their nutritional values are well known mainly to local people, but there is not a validation of their bioactive compounds in research studies carried out to date. This study explores the functionality of these local natural plants by informing consumers about their content of polyphenols and other antioxidants.

Total phenolic and antioxidant capacity of the 17 analyzed herbs and wild greens are presented in Table 1.

Mean values of the total phenolic content ranged from 0.18 to 2282.80 mg GAE/g of sample. More specifically, *Hypericum perforiatum* L. and *Hypericum perforatum* L., *Salvia* spp., and *Sideritis* sp. had the highest phenolic compound content (2282.80 ± 453.64 , 946.91 ± 126.21 , and 819.03 ± 247.64 mg GAE/g of sample, respectively) while the lowest was found in *Pistacia lentiscus* var. *chia*, *Panocratium maritimum*, and *Crataegus azarolus* L. (0.18 ± 0.05 , 1.09 ± 0.31 , and 1.32 ± 0.52 mg GAE/g of sample, respectively). The decreasing order of total phenolic content was *Hypericum perforiatum* L. and *Hypericum perforatum* L. > *Salvia* spp. > *Sideritis* sp. > *Melissa officinalis* L. > *Mentha spicata* > *Thymbra capitata* > *Origanum* sp. > *Crithmum maritimum* L. > *Salicornia europaea* L. > *Thumys* sp. > *Ocimum basilicum* L. > *Rosmarinus officinalis* L. > *Matricaria chamomilla* L. > *Otanthus maritimus* > *Crataegus azarolus* L. > *Panocratium maritimum* > *Pistacia lentiscus* var. *chia*. These results are consistent with those obtained by Gutiérrez-Grijalva et al., which stated that a specific part (leaves) of the plant *Origanum* sp. resulted in a phenolic content of 79–147 mg GAE/g of DW extract [21].

Recent publications from Sotiropoulou N.S. et al. concluded the impact of the extraction temperature results in a variation of the phenolic content of *Matricaria chamomilla* L. [22]. As stated in their study, the higher the temperature (80 °C to 100 °C) the greater the phenolic compounds. Therefore, this could be a possible explanation for the low values that *Matricaria chamomilla* L. presented in our study.

Table 1. Total phenolic and antioxidant contents of the 17 studied herbs and wild greens.

Plant Sample	Total Phenol (mg GA/g of Sample)	Total Antioxidant (mmol Fe ²⁺ /L)
<i>Ocimum basilicum</i> L.	16.51 ± 3.13	7.42 ± 0.71
<i>Matricaria chamomilla</i> L.	9.57 ± 3.12	5.31 ± 0.81
<i>Crataegus azarolus</i> L.	1.32 ± 0.52	0.57 ± 0.08
<i>Thymbra capitata</i> L.	36.68 ± 8.64	12.29 * ± 2.07
<i>Rosmarinus officinalis</i> L.	10.78 ± 2.86	4.52 ± 1.47
<i>Melissa officinalis</i> L.	61.70 ± 15.31	30.58 * ± 5.15
<i>Mentha spicata</i>	39.61 ± 31.83	26.97 * ± 11.28
<i>Pistacia lentiscus</i> var. <i>chia</i>	0.18 ± 0.05	0.06 ± 0.04
<i>Origanum</i> sp.	32.09 ± 12.72	12.11 ± 3.97
<i>Thymus</i> sp.	18.42 ± 2.65	4.40 ± 2.22
<i>Pancreatium maritimum</i>	1.09 ± 0.31	0.18 ± 0.03
<i>Otanthus maritimus</i>	1.33 ± 0.38	0.47 ± 0.06
<i>Crithmum maritimum</i> L.	27.09 ± 13.82	0.28 ± 0.18
<i>Salicornia europaea</i> L.	21.32 ± 6.39	0.12 ± 0.05
<i>Salvia</i> spp.	946.91 * ± 126.21	4.72 ± 0.61
<i>Hypericum perforatum</i> L. and <i>Hypericum perforatum</i> L.	2282.80 * ± 453.64	8.76 ± 1.88
<i>Sideritis</i> sp.	819.03 * ± 247.64	3.96 ± 0.91

Statistically significant differences were observed between the samples of the different food extracts in respect to their antioxidant capacity ($p < 0.05$) and their phenolic content ($p < 0.05$). The values with asterisk (*) represent the highest values of phenolic compounds and antioxidant activity, statistically significant compared with the rest of the plants.

The antioxidant capacity ranged from 0.06 to 30.58 mmol Fe²⁺ /L. *Melissa Officinalis* L., *Mentha spicata*, and *Thymbra capitata* L. displayed the greatest antioxidant capacity (30.58 ± 5.15, 26.97 ± 11.28, and 12.29 ± 2.07 mmol Fe²⁺ /L), whereas *Pistacia lentiscus* var. *chia*, *Salicornia europaea* L., and *Pancreatium maritimum* the displayed lowest (0.06 ± 0.04, 0.12 ± 0.05, and 0.18 ± 0.03 mmol Fe²⁺ /L). The decreasing order of total antioxidant capacity was the following: *Melissa officinalis* L. > *Mentha spicata* > *Thymbra capitata* L. > *Origanum* sp. > *Hypericum perforatum* L. and *Hypericum perforatum* L. > *Ocimum basilicum* L. > *Matricaria chamomilla* L. > *Salvia* spp. > *Rosmarinus officinalis* L. > *Thumys* sp. > *Sideritis* sp. > *Crataegus azarolus* L. > *Otanthus maritimus* > *Crithmum maritimum* L. > *Pancreatium maritimum* > *Salicornia europaea* L. > *Pistacia lentiscus* var. *chia*. Comparing these results with the works of the literature, Boneza M.M. et al. concluded that *Melissa officinalis* presented the greatest values of antioxidant capacity, due to the high consistency in rosmarinic acid, which is known for its antioxidant properties [23]. Furthermore, from the above results, it is observed that *Origanum* sp. and *Mentha spicata* showed a high antioxidant capacity. This is in line with Saija A. et al. and Goncalves S. et al., where they found that the antioxidant properties of the herb extracts are due to the high content of rosmarinic acid in the aerial parts of the plant (35.63 ± 2.09, 23.53 ± 0.47 mmol Fe²⁺ /L, respectively) [24,25]. It is important to mention that in this study all solutions were water-soluble and during the extraction no organic solvents were used. However, according to Koutelidakis E.A. et al. and Sultana B. et al., it has been suggested that the use of organic solvents can benefit the extraction process, but in this study water-soluble solvents were selected so as to be used afterward for the fortification of functional food products such as yogurt by the form of water-soluble extracts [26,27]. Thus, the water-soluble solvents could be a possible limitation of this study, and organic solvents could be used to have more accurate results in the total compounds of medicinal plants.

Overall, total antioxidant values higher than 0.87 mmol/L, as described in the study of Ulewicz-Magulska B. and Wesolowski M., can be considered applicable for the use as natural food additives in functional foods [28]. For example, Shori B.A. mentioned that fortified yogurt with extracts of medicinal plants, such as rosemary and oregano, significantly ($p < 0.05$) increased the FRAP value in yogurt [29]. Therefore, this study could provide beneficial practical applications of the use of natural herbs and wild greens extracts to different types of foods (such as dairy products). Further investigation of the geographical variation and/or methods of determination of bioactive compounds (in vitro and in vivo) are necessary to better understand the differences in the bioactivity of the medicinal plants. In addition, further assessment of a larger variety of local edible medicinal plants from the North Aegean should be explored in future research.

4. Conclusions

In conclusion, in this study, the determination of the polyphenolic content and antioxidant capacity of the edible medical plant extracts indicates that the studied foods could be a significant source of antioxidants. Therefore, it can be suggested that the above herbs and plants could be applicable in the fortification of food products, such as in yogurt, and the formulation of sustainable food production by enhancing the Mediterranean diet principles.

Author Contributions: Conceptualization, A.K. and A.E.K.; methodology, C.K., K.A., A.K., P.P. and P.B.; validation, A.K. and A.E.K.; formal analysis, C.K. and A.K.; investigation, C.K., A.K., P.P., P.B. and A.K.; resources, A.E.K.; data curation, C.K. and A.K.; writing—original draft preparation, C.K., K.A., A.K., P.P., P.B., A.K. and A.E.K.; writing—review and editing, C.K., A.K. and A.E.K.; visualization, A.K. and A.E.K.; supervision, A.E.K.; project administration, A.E.K.; funding acquisition, A.E.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Operational Program “Competitiveness, Entrepreneurship and Innovation” (EPAnEK), under the project “Infrastructure of Microbiome Applications in Food Systems-FoodBiomes”.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Table 1.

Conflicts of Interest: The authors declare no conflict of interest.

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