Antioxidant Activity of Cretan Herbs and Herbal Combinations

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RESEARCH

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ABSTRACT

Objective: Herbs are an integral component of the Cretan dietary culture, which has been linked to lower risk of coronary heart disease. The health promoting effects of many herbs have been attributed to the presence of antioxidants. The aim of the present study was to evaluate the antioxidant activity of five herbs, native to Crete: Matricaria recutita, Salvia fruticosa, Origanum majorana, Origanum dictamnus, Sideritis syriaca, and four of their most commonly consumed binary combinations.

Methods: Total phenolic contents were measured by the Folin-Ciocalteu method. Antioxidant capacity of the herbal extracts was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) methods.

Results: The herbal combinations exerted high free radical scavenging activity and reducing power. The results of this study are indicative of synergistic antioxidant interactions between the respective herbs and underline the need for further research on the antioxidant potential of Cretan herbal combinations.

Conclusions: Combinations of the Cretan herbs sage with dittany, mountain tea, chamomile, or marjoram may synergistically enhance total antioxidant potential, and therefore may yield more positive health effects.

Key Words: Phenolic content, antioxidant activity, DPPH, FRAP, herbal extracts.

ABBREVIATIONS

Word	Abbreviation
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	ferric reducing antioxidant power
gallic acid	3,4,5-Trihydroxybenzoic acid
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
SD	standard deviation

INTRODUCTION

Crete is known since ancient times for its medicinal plants and herbs [1]. It has one of the richest and most interesting ecosystems in Europe, classified as a UNESCO Biosphere Reserve [2, 3]. The use of cretan herbs for treatment of ailments has its origins in the Bronze Age [1].

Herbs constitute an integral component of the Cretan dietary culture, which has attracted increasing scientific attention, ever since the “Seven Countries Study” reported markedly lower mortality rates for the Cretan cohort [4]. Cretan herbs have been traditionally consumed, as infusions of single herbs or herbal combinations, and

have been valued for their healing properties, especially for the treatment and prevention of common cold and influenza [5].

According to the recent ethnopharmacological survey, which included 103 medicinal plants traded in herbal markets of Peloponnisos (Greece), the indigenous plants chamomile and sage were among the herbs with the highest use value [6]. The medicinal uses of sage include dyspepsia, ulcer, diarrhea, hypotension, tonsillitis, laryngitis, influenza, common cold, asthma, headache, migraine, depression and dysmenorrhea. Dittany is used as a calmative, a diuretic, a stimulant of the immune system, and for liver detoxification. It is also used in the treatment of stomach disorders, influenza, common colds, headache, and dysmenorrhea. Marjoram is used to treat flatulence, stomach ache, nausea, common cold, headache, insomnia, vertigo and stress. Mountain tea is used as a cardiotonic and for the treatment of gastroenteritis, dyspepsia, stomach ache, diarrhea, and common cold. Chamomile is used to treat colic, dyspepsia, diarrhea, flatulence, stomach disorders, constipation, ulcer, stress, insomnia and migraine.

Modern research has shed light on the medicinal properties of herbs and it is now widely accepted that their therapeutic effects are largely due to their high content of bioactive constituents, such as phenolic compounds, alkaloids, saponins and terpenoids among others [7]. Several classes of bioactive compounds exert antioxidant activity either as primary antioxidants by scavenging free radicals, or as secondary antioxidants by preventing formation of free radicals [8]. Phenolic compounds (e.g. phenolic acids, coumarins, flavonoids, tannins, lignans, and lignins) are the largest group of phytochemicals and one of the most important sources of antioxidants in the human diet [9]. However, various types of antioxidants may act synergistically, thereby enhancing total antioxidant capacity [10]. Synergistic interactions may also occur in herbal combinations between antioxidant constituents.

Over recent years, a number of epidemiological studies have demonstrated that long-term consumption of herbs, as part of a balanced diet, may improve antioxidant status, reduce oxidative stress and protect against several non-communicable diseases such as cancer, diabetes type II, and autoimmune diseases [7, 11, 12]. Most of these health promoting properties have been linked to antioxidant compounds.

The aim of the present study was to evaluate the in vitro antioxidant activity of five herbs that are native to the mountains of Crete: Matricaria recutita (chamomile), Salvia fruticosa (sage), Origanum majorana (marjoram), the endemic species Origanum dictamnus (dittany) and Sideritis syriaca (mountain tea), as well as, four of their most commonly consumed binary combinations.

MATERIALS AND METHODS

Materials

Folin–Ciocalteau reagent, 3,4,5-Trihydroxybenzoic acid (gallic acid), rutin (95%), DPPH, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich (Germany). Methanol, ethyl acetate and ascorbic acid were purchased from Merck (Germany). All other chemical reagents used were obtained from Sigma-Aldrich (Germany). The chemical reagents and solvents were of analytical grade.

Sample Preparation

Five pre-packaged dry Cretan herbs: chamomile, sage, marjoram, dittany, and greek mountain tea, were acquired from the local market of Chania, Crete, Greece. 3g of each herb separately was Soxhlet extracted with 70 ml of methanol (100%) for 16 h. The methanol herb extracts were evaporated at 40 °C and the residue obtained was redissolved in methanol to a final volume of 7 ml and then centrifuged at 8000 rpm for 15 min. Finally, the stock solutions of extracts were prepared at a concentration of 20 mg/mL, using methanol. For herb pair analysis, the methanol extracts of two herbs were mixed in equal proportions. Sage was chosen as the standard component of all herb pairs, due to its use as the basic constituent of

many traditional Cretan herbal mixtures. All herbal extracts were stored at 4 °C.

Determination of Total Phenolic Content

The Folin-Ciocalteu colorimetric method was used for the measurement of total phenolic content of each sample [13]. Briefly, 100 µl of sample was mixed with 1 ml of the Folin–Ciocalteu reagent (diluted ten-fold) and incubated at room temperature for 5 min, and then 1 ml of a 10% Na_2CO_3 solution was added to the mixture. The absorbance was measured at 765 nm on a UV/visible spectrophotometer (Chemito SPECTRASCAN UV 2600PC), after a 90 min incubation at room temperature. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. Results were expressed as mg of gallic acid per gr of herb dry weight.

Determination of Antioxidant Activity by the DPPH Assay

The DPPH radical scavenging activity assay was carried out as previously described by Rathee and colleagues with slight modifications [14]. Briefly, 0.1 ml samples of various herb concentrations in methanol were added directly to 3.9 ml of a DPPH solution in methanol (0.1 mM). The mixture was immediately shaken vigorously for 10 s on a vortex mixer, and then kept at 37 °C for 30 min. The absorbance was measured at 517 nm and the radical scavenging activity (% DPPH) was calculated by the percentage of DPPH that was scavenged using the following formula:

$$\%DPPH = (AB - AS)/AB)x100$$

Where: AS is the absorbance of the DPPH solution after reacting with the sample at a given concentration and AB is the absorbance of the DPPH solution with a methanol blank instead of a sample.

Determination of Antioxidant Activity by the FRAP Assay

The FRAP assay was performed as described by Benzie & Strain with slight modifications [15]. FRAP reagent was prepared daily by mixing acetate buffer (300 mmol/L, pH=3.6), TPTZ solution (10 mmol/L in 40 mmol/L HCl) and 20 mmol/L $FeCl_3 \bullet 6H_2O$ solution in proportions of 10:1:1. The mixture was incubated at 37 °C for several minutes. 0.1 ml samples dissolved in methanol were added directly to 3.9 ml of FRAP reagent. The absorbance of the reaction mixture was then measured at 593 nm after 10 min. The calibration curve was plotted by injecting standard solutions of ascorbic acid concentrations from 0.1 to 1.2 mmol/L. Results were expressed as mM ascorbic acid per g of herb dry weight.

Statistical Analysis

All measurements were carried out in triplicate and values were expressed as mean ± standard deviation (SD). Data were processed using Microsoft Excel 2007.

RESULTS

The total phenolic contents of the herbal extracts were calculated according to the standard curve shown in Figure 1. Table I presents total phenolic contents and antioxidant activities of herbal extracts derived from FRAP and DPPH assays. The highest level of phenolics was found in marjoram while the lowest was found in chamomile. The greatest FRAP value was recorded for marjoram and the lowest was recorded for chamomile. Sage exhibited the highest % DPPH radical scavenging potency, while chamomile exhibited the lowest. The antioxidant activities of the herb pair extracts derived from FRAP and DPPH assays are shown in Figures 2 and 3, respectively.

DISCUSSION

The present study was undertaken to evaluate the phenolic content and antioxidant activity of five popular Cretan herbs, as well as, some of their most commonly consumed binary combinations. Since antioxidants act via several mechanisms, both radical scavenging activity and ferric reducing antioxidant power of the herbal extracts were measured.

Total phenolic contents of the five single herbal extracts decreased in the following order: marjoram > sage

> mountain tea > dittany > chamomile. Although a direct comparison with data obtained in previous studies is not possible due to differences in the extraction procedures applied, these results are in agreement with those reported by Proestos and colleagues, who found a similar order of phenolic levels:sage > mountain tea > dittany > chamomile [16]. Also, Chrpova et al. (2010) demonstrated that marjoram extract had higher phenolic content compared to sage [17]. The antioxidant capacity, as evaluated by FRAP, decreased in the same order as that of the total phenolic content, indicating that phenolic compounds have a major contribution to reducing antioxidant power of these herbs [18].

The antioxidant activity of the herbal extracts, as evaluated by DPPH, was in the order: sage > marjoram > dittany > mountain tea > chamomile. The difference in the ranking order is most likely due to differences in the methods used, since FRAP evaluates ferric reducing ability of the antioxidants, whereas DPPH evaluates the free radical scavenging ability of antioxidants, and is sensitive to steric hindrance effects [19]. However, the higher % DPPH value of sage compared to marjoram has also been demonstrated by Mariutti and colleagues [20]. Interestingly, according to Lionis and colleagues extracts of Salvia fruticosa (sage) have high antioxidant capacity, as documented by decreased lipid peroxidation [5].

All herbal combinations exerted high radical scavenging activity and reducing antioxidant power, even the combination with chamomile, which is known to have moderate in vitro antioxidant activity [21]. The highest % DPPH radical scavenging values were recorded for sage with dittany and sage with chamomile, whereas the highest FRAP values were recorded for sage with mountain tea and sage with dittany. The antioxidant activities resulting from the herbal combinations are indicative of synergistic antioxidant interactions between the respective herbs. Mechanisms underlying antioxidant synergistic interactions may involve regeneration of primary antioxidants, metal chelation, sacrificial oxidation, etc. [22, 23].

This is the first study to our knowledge to measure the antioxidant activity of Cretan herbal combinations. Yet, infusions prepared by Cretan herbal combinations are commonly consumed as remedies to combat cough, sore throat, common cold and influenza [5, 24, 25]. Recent studies demonstrate the effectiveness of a combination of thyme, sage and dittany on the treatment of upper respiratory tract infections [25, 26], providing evidence on the synergistic action of Cretan herbs.

The present study has some limitations which should be acknowledged. First of all, only methanolic herbal extracts were evaluated, therefore values on the phenolic content and antioxidant potential may greatly differ in herbal infusions. Also, only in vitro methods of assessing antioxidant activity were applied, therefore the clinical relevance of these findings is uncertain. Further research is required to investigate synergistic antioxidant effects in Cretan herbal combinations and to assess their therapeutic effectiveness.

However, many traditionally used herbs demonstrate significantly better pharmacological effects when used in combination than when used alone [22, 27]. Therefore, it seems likely that the diversity of antioxidants found in herbs, their multiple individual functions and their ability to act in a complementary and synergistic manner are crucial to their beneficial effects on human health. Moreover, the fact that Cretan herbs comprise an integral component of a dietary culture, which has been linked to lower risk of coronary heart disease and greater longevity [4, 28], underlines the significance of exploring the potential health benefits of Cretan herbs and their combinations.

CONCLUSIONS

Combinations of the Cretan herbs sage, dittany, mountain tea, chamomile, and marjoram exerted high free radical scavenging activity and reducing power, suggesting that these herbs may synergistically enhance total antioxidant potential, and therefore may yield more positive health effects.

Conflicts of interest

The authors declare that they have no competing interests.

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PEER REVIEW

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TABLE

Table 1. Total phenolic content, % DPPH and FRAP values of herbal extracts

Herb	**Total phenolic content** (mg gallic acid/gr dry weight)	**FRAP** (mM vitamin C/gr dry weight)	**% DPPH**
Chamomile	0.46±0.08	0.588±0.002	4.60±0.72
Dittany	4.46±0.32	0.653±0.009	10.19±0.58
Mountain tea	4.78±0.13	0.664±0.005	6.50±0.45
Sage	10.96±0.79	0.743±0.012	30.58±0.62
Marjoram	16.57±0.34	0.802±0.003	16.41±0.54

FIGURES

Figure 1: Standard calibration curve of Gallic acid for the determination of total phenolic content.

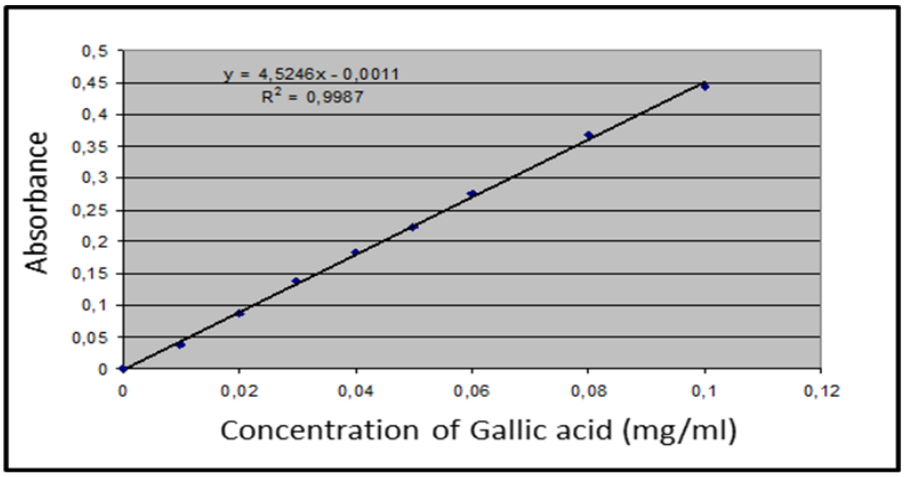

Figure 2: FRAP values of herb pairs along with respective single herbs. Data are shown as mean ± SD, n=3.

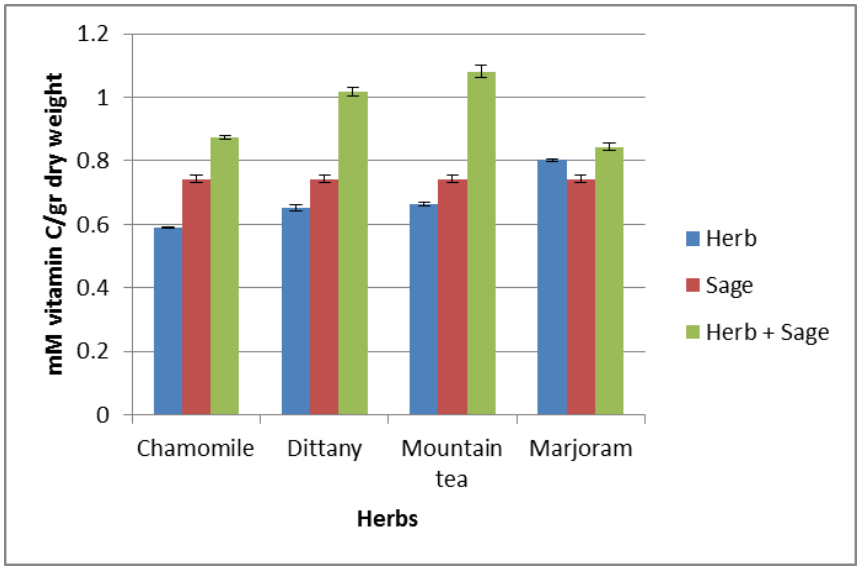

Figure 3: % DPPH radical scavenging activity of herb pairs along with respective single herbs. Data are shown as mean ± SD, n=3.