

ANTIOXIDANT CONTRIBUTION OF LAVENDER (*Lavandula angustifolia*), SAGE (*Salvia officinalis*), TILIA (*Tilia tomentosa*) AND SIDERITIS (*Sideritis perfoliata*) BEVERAGES PREPARED AT HOME.

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Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

CITATIONChristos Kontogiorgis, ANTIOXIDANT CONTRIBUTION OF LAVENDER (*Lavandula angustifolia*), SAGE (*Salvia officinalis*), TILIA (*Tilia tomentosa*) AND SIDERITIS (*Sideritis perfoliata*) BEVERAGES PREPARED AT HOME. (2018) SDRP Journal of Food Science & Technology 3(4)**ABSTRACT**

Background: Nature provides us with all the necessary ingredients to lead a healthy life, while data from ancient civilizations and scientific discoveries support the fact that the use of aromatic plants and herbs due to their therapeutic properties is a neglected healthy habit.

Methods: This study examines the antioxidant activity in domestic preparation of four herb beverages Lavender (*Lavandula angustifolia*), Sage (*Salvia officinalis*), Tilia (*Tilia tomentosa*) and Sideritis (*Sideritis perfoliata*) aiming to investigate the presence of antioxidants in the plants and to define which domestic preparation method of the beverage (decoction/infusion) ensures better antioxidant capacity. In the experimental procedure, herbal decoctions and infusions (boiling time 5 minutes) were extracted with 4 different solvents of increasing polarity (petroleum ether, diathylether, ethyl acetate, butanol). This process resulted to 10

samples from each herb beverage. Each of them was examined for the TPC via the Folin - Ciocalteu method, the interaction with the cationic radical ABTS, the free stable radical DPPH for the determination of antioxidant capacity, the hydroxyl radical scavenging ability, the inhibition of lipid peroxidation of linoleic acid and finally the acceptability of each beverage with respect to the manner of preparation through sensory evaluation in a total number of 40 random volunteers.

Results: The results exhibit the characteristics represented by each herb. Comparison and classification between them was also performed. The antioxidant characteristics of the herbs were affected both by the preparation method as well as the extraction solvent.

Conclusions: All herbs exhibited high antioxidant capacity. However, not all were positively evaluated during the organoleptic evaluation which raises the question of whether these beneficial herbs would be

included as beverages in every-day diet. Our research exhibited the capacity of these herbs as well as the need for organoleptic evaluation to be included in research.

Abbreviations: TPC: Total Phenolic Content, TCM: Traditional Chinese Medicine, TEAC: Trolox-Equivalent Antioxidant Capacity, OD: Optical Density, GAE: Gallic Acid Equivalent, HAT: Hydrogen Atom Transfer

Key words: Beverages, Antioxidants, Lipid Peroxidation, Phenolic Content, Lipoxigenase

INTRODUCTION

It is widely known that many natural products are highly beneficial for good health. Ever since ancient times, and much prior to scientific thinking and verification, humans would use plant-based remedies for treatment and prophylaxis.[1] Mediterranean diet is associated with the prevention and treatment of chronic diseases and longevity, by also being a standard way of lifestyle. Beverages from herbs of Mediterranean, have an important role in the Mediterranean diet pyramid, both nutritionally and culturally.

Studies have shown that following the Mediterranean diet(-s) can lead to a 14% lower mortality rate, while there are numerous studies to support this beneficial diet in the framework of health promoting and disease prevention or treatment.[2–4] Additionally, Mediterranean-style diets are close to American Heart Association dietary guidelines.[5] Plant food bioactive compounds have been related to beneficial effects for a number of health conditions.[6–8] It is known that the Mediterranean diet's composition contains large amounts of antioxidant vitamins (vitamins C, A, E and beta-carotene) that have a beneficial effect in the treatment of various diseases, such as those of the cardiovascular system, in type 2 diabetes, cancer and obesity while there are also findings that it can even reduce the risk of Alzheimer's and Parkinson's.[9–11]

As the base of the Mediterranean diet, fruits, vegetables, grains, seeds etc. have been the centre of attention for researchers worldwide. Although many spices and herbs, rich in flavonoids, have been used for years in medicine, the pharmaceutical usage of

plant compounds had faced its ups and downs over the evolution of medicine, with a 2008 estimate stating that 25% of the commonly used medicines contain compounds isolated from plants.[12] However, the search for new molecules, has taken a route where the science of ethnobotany and ethno-pharmacognosy are being used as guide to lead the chemist towards different sources and classes of compounds.[13]

Flavonoids are now known for their antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities.[14–18] Fruits and vegetables are the main dietary sources of flavonoids for humans, along with tea and wine. However, a clear understanding of the mechanisms of action of flavonoids, either as antioxidants or modulators of cell signalling, and the influence of their metabolism on these properties are key to the evaluation of these potent biomolecules as anticancer agents, cardioprotectants, and inhibitors of neurodegeneration.[19]

Although information on the working mechanisms of flavonoids is still not understood properly, increased flavonoid consumption is associated with reduced mortality from coronary heart disease in women and men and with reduced risk of coronary heart disease by 38% in postmenopausal women.[20–23] It seems that the combination of food and biological interactions of the different components of the Mediterranean diet provides important health benefits, with high antioxidant content, contributing significantly to the antioxidant value of this diet model.[9, 24–28]

The world of plants includes some 350,000 species, with 18,000 classified as *aromatic* and many classified as therapeutic due to substances they contain with therapeutic properties. Most herbs grown in Greece belong to the family of Labiatae (*Lamiaceae*). [29–35]

Lavender (*Lavandula angustifolia*) was used in ancient Rome and North Africa for perfuming and in TCM, in order to treat infertility, infection and stress, while in Arabic medicine it was used to treat stomach pain and kidney problems. It was widely used as an

aphrodisiac during the Victorian era while later it has been classified as antidepressant, antispasmodic, anti-flatulence antiemetic, diuretic and tonic.[29,36–40] Its great appeal and commercial value was confirmed again when it was named Herb of the Year in 1999 by Herb Growing and Marketing Network in the United States of America.[16-17]

To date, Lavender (*Lavandula angustifolia*) continues to excite the scientific community through significant results both on *in vitro* testing and in clinical trials.[41–44]

Sideritis (*Sideritis perfoliata*), widely known as “mountain tea”, has been used for centuries as a homemade beverage due its soothing characteristics. It is widely used as aromatic and warming drink, for its anti-inflammatory properties. It is beneficial to the blood vessels of the heart and also has gastroprotective properties and is used in rheumatic diseases, diarrhea and dyspepsia.[38,45–48] Aside of its antioxidant characteristics, Sideritis has also been intensely researched for its effects on glucose and lipid disorders, while literature also mentions an interest on psychopharmacological effects.[49–52]

Tilia (*Tilia tomentosa*) has been known for their analgesic, antibacterial and anti-infective characteristics. Furthermore, studies have shown that Tilia can contribute as antispasmodic, antiviral and also auxiliary the circulatory system, while literature also mentions efficacy as adjuncts to adaptogens.[53–56]

Sage (*Salvia officinalis*) has been traditionally used to treat sweating and menopausal hot flushes, as well as to alleviate associated menopausal symptoms and as a general tonic, while in folk medicine, it has been used for the treatment of different kinds of disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycaemia.[57-58] Additionally, *S. Lavandula efolia* Vahl. (Spanish sage) extracts and constituents have demonstrated anticholinesterase, antioxidant, anti-inflammatory, oestrogenic and CNS depressant (sedative) effects all of which are relevant to the treat-

ment of Alzheimer's disease (AD).[59]

Several studies aimed to the determination of the antioxidant activity of these herbs using a variety of methods and they conclude that herb extracts show strong antioxidant activity.[36-37,56,60–64] Furthermore, the impact of boiling conditions has often been examined, showing that the preparation conditions of a beverage influence its characteristics.[30,65–69]

This study examines the antioxidant capacity of beverages of Lavender (*Lavandula angustifolia*), Sage (*Salvia officinalis*), Tilia (*Tilia tomentosa*) and Sideritis (*Sideritis perfoliata*) and aims to point out the influence of preparation method in household conditions, on the antioxidant capacity. According to previous studies on plant extracts, there is a relation between the solvents' polarity and free radical scavenging activity also related to the presence of phenolic acids, although a lot of research is related to the essential oils of the plants.[37, 70–72] In this research, we examine this aspect but also attempt a comparison among those four beverages considering their antioxidant characteristics examining them separately *in vitro*.

Materials and Methods

For the experimental procedure, we used dried herbs. Namely: **Lavender** (flowers), **Sage** (leaves) and **Tilia** (leaves and flowers) were procured from Myrtis nurseries which were collected and dried in Polykarpi Pozar Greece. **Sideritis** (all parts) was collected and dried in Pertouli Trikala Greece (packs of 50 gr).

Solvents and Solutions: Petroleum ether (PE), Diethyl ether (DE), Ethyl acetate (EA), 1-butanol (BuOH), 1,1-Diphenyl 2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2'-azobis-(2-amidinopropane)-dihydrochloride (AAPH), Dimethyl sulfoxide (DMSO), Ethylenediaminetetraacetic acid (EDTA), lipoxigenase type I-B (soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis, MO, USA). All chemical reagents were of analytical grade. Nash solution was obtained by adding 45g ammonium acetate, 0.9 ml acetic acid and 0.6 ml acetyl acetone in 100ml H₂O. Regarding the preparation methods of the bever-

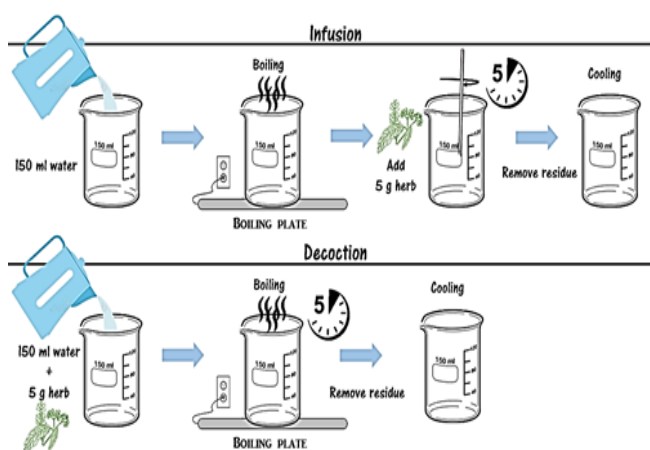
ages, the boiling time was 5 minutes, in order to prepare the infusions and decoctions from each herb. Specifically, for the preparation of the infusion, the water was boiled for 5 minutes and then added with the 5g of dried herb on the contrary to the decoction where water and 5g of dried herb boil outset together for 5 minutes. In both cases the solution is filtered so as to separate the solid residue, which were subjected to extraction with four different solvents of increasing polarity.

2.1. Beverage's Preparation

Distinguishing the difference between Infusion and Decoction. According to Merriam-Webster *Infusion* is a drink made by material using liquid (most commonly hot water) following these steps: (1) the liquid (usually water) was boiled (2) the material is added for a certain period under stirring and (3) the liquid extract was filtered. (Scheme 1)

Decoction is a different procedure following these steps: (1) the material was boiled in parallel with the liquid (usually water) and (2) the extract was filtered (Scheme 1). [73-74]

In order to be clear regarding the preparation method, as its significance would influence the outcomes of this study as described in the introduction, we have developed a schematic presentation of the process, shown in Scheme 1.



Scheme 1, Distinguishing the difference between Infusion and Decoction.

For the preparation of both procedures 5g of each herb were used in 150ml of distilled water and they left boiling on a hearth for 5 minutes. After 5 minutes, solid residue is removed and the mixture is left to cool.

Each part of the preparation took place separately for each herb following the exact same process. The mixture resulting from each process is subjected to extraction using solvents of increasing polarity: Petroleum ether (PE), Diethyl ether (DE), Ethyl acetate (EA) and 1-Butanol (BuOH). Each extract is evaporated, dried and the solid residue stored in the fridge. The whole procedure is repeated three times for each herb and the solid residues are stored in vials. We ultimately received overall 10 vials of solid residues, whose weights were measured in grams on an analytical balance and preserved sealed at 4°C for further analysis.[73]

2.2. Determination of Total Phenol Content

The total phenol content of the obtained fractions was determined using the method of Zheng and Wang with a few modifications: 1 mg of dry residue was dissolved in 1ml DMSO or H₂O.[75] 10µl of this solution was transferred to a volumetric flask to which 50µl of Folin-Ciocalteu phenol reagent and 150µl of 20% sodium carbonate solution were added. The mixture was shaken thoroughly and kept for 120 minutes at 25-30°C, in absence of light. The absorbance of the blue color formed was measured at 765nm. The concentration of total phenol compounds for each extract was calculated on the basis of a standard curve obtained using gallic acid as the standard (twelve serial-2-fold dilutions to give a range of 0.01– 0.001mg/mL in triplicate). Results were expressed as concentration of phenolic mg/L of each solvent compared to the different boiling times.

2.3. Antioxidant capacity

2.3.1. Evaluation of TEAC by ABTS

A method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma

antioxidants. The pre-formed radical mono cation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants.[76] The Trolox Equivalent Antioxidant Capacity (TEAC) assay is based on the scavenging of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical converting it into a colorless product. The degree of decolorization induced by a compound is related to that induced by Trolox, giving the TEAC value.[77]

In this assay, ABTS is converted to its radical cation by addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734nm. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The reaction was monitored spectrophotometrically. For each sample, we prepared 3 mixtures which contained a 50µl sample -from the stock solution of the sample (approximately 1 mg in 1 ml DMSO/H₂O and 1950µl ABTS (7mM). The mixture was shaken thoroughly and kept for 6 minutes at room temperature before measuring the absorbance.[21,76,78–81]

2.3.2. Interaction with Stable Free Radical of DPPH

DPPH assay test is very useful in the micromolar range. 1,1-Diphenyl-picrylhydrazyl is a stable free radical and antioxidants react with it and reduce it to DPPH-H while the absorbance decreases. The radical's solution is purple color which turns yellow when the radical is scavenged, while the degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability and can be followed spectrophotometrically (517nm).[21,32,64,81–83]

A 20µl sample from the stock solution of the sample (2.5mg/1ml DMSO) were dissolved in absolute ethanol to a final volume of 1 ml and then added to 1 ml DPPH (0.1mM, in absolute ethanol). The reaction mixture was kept at room temperature. The OD of the solution was measured at 517nm, after 20 minutes and 60 minutes. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction values

were determined and compared to appropriate standards.

$$\% \text{Reduction} = \frac{\text{control OD}(\text{mean}) - \text{sample OD}(\text{mean})}{\text{control OD}(\text{mean})} * 100$$

2.3.3. Competition of the tested compounds with DMSO for hydroxyl radicals

The hydroxyl radicals generated by the FeCl₃/ascorbic acid system, were detected by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (200µl), FeCl₃ (150µl), DMSO (200µl) in phosphate buffer (240µl, pH 7.4), the tested compounds (10µl) and ascorbic acid (150µl). After 30 minutes of incubation (37°C) the reaction was stopped with the addition of CCl₃COOH (17.5% w/v) and 1ml of Nash solution was inserted. After 10 minutes of incubation (60°C) the absorbance of the samples was measured at 734nm.[84–86]

2.3.4. Inhibition of Linoleic Acid Lipid Peroxidation

The water soluble azo compound AAPH is used as a free radical initiator for *in vitro* studies of free radical production. Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution is monitored at 234nm. An amount of 10µl of the 16mM linoleic acid sodium salt solution was added to the UV cuvette containing 0.93ml of 0.05M phosphate buffer, pH 7.4, pre-thermostated at 37°C. The oxidation reaction was initiated at 37°C under air by the addition of 50µl of 40mM AAPH solution. Oxidation was carried out in the presence of aliquots (10µl) in the assay without antioxidant, and lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. [73,75,87-88]

2.3.5. Organoleptic Evaluation

Much of the potential of sensory information for understanding primate feeding has been ignored because

the subject is usually approached from a nutritional perspective rather than a sensory one.[89]

Organoleptic properties are the aspects of food, water or other substances; that a person experiences via the senses-including taste, sight, smell, and touch. The organoleptic properties of food have a determining effect on consumption and commercial success. There are four major types of organoleptic testing: (1) Difference testing, which identifies differences from one sample to another, (2) Sensitivity testing, which assesses stimulus-response dynamics, (3) Preference testing, which involves 'like' or 'dislike' feedback in relation to the sample, (4) Descriptive testing, which dwells on rated characteristics.[90–94] There is limited information on the effect of the compounds of a beverage on its sensory characteristics and research usually focuses on its quality on matters other than acceptability.[90,95-96]

Due to lack of extent literature on the questionnaire requirements a questionnaire was created by making some adjustments to questionnaires used in

previous studies.[90,94,96] The testing was descriptive, with mono-polar and bipolar scales for each required characteristic. Samples of each herb beverage were prepared at the same time (both decoction and infusion) and given to the testers at the same time after cooling. Before the evaluation, the questionnaire was explained to the panellists and each herb beverage was evaluated at a different time and each time from different panellists so as to avoid partial comparison in between herbs.

3. Results

3.1. Isolation of organic extracts

After the preparation of each herb beverage, samples were extracted with a series of organic solvents with increasing polarity. Solid residues' weight after the extraction and evaporation are shown in **Table I**. The results suggest that the solvents' polarity influenced the residues weight with an increasing tendency, while there are not significant differences regarding the preparation method.

Table I: Solid Residue (g) after the Extractions

	SOLVENTS	PE	DE	EA	BuOH	H ₂ O
Solid residue (g)	Lavender Infusion	0.0480	0.0658	0.0760	0.2165	1.4945
	Lavender Decoction	0.0564	0.0622	0.0641	0.1401	1.9798
	Sideritis Infusion	0.1729	0.1834	0.2918	0.2134	1.7236
	Sideritis Decoction	0.1548	0.1739	0.1997	0.2458	1.8931
	Tilia Infusion	0.0357	0.0383	0.0391	0.1627	1.0827
	Tilia Decoction	0.0166	0.0211	0.0212	0.2878	1.0640
	Sage Infusion	0.3228	0.0526	0.0732	0.1054	1.1046
	Sage Decoction	0.5020	0.1012	0.4223	0.0715	1.0697

In all cases the solvents polarity had an effect on the residue's quantity, favouring the most polar solvents in most cases except the case of Sage in which resulted to an abnormal distribution between solvents in the decoction samples. Lastly, in all cases the decoction extracts performed better in the residue giving part, which gave us the first hint on the debate of the best preparation method of the beverage.

3.2 Determination of TPC

Phenolic compounds are widely distributed in plant kingdom and they present significant antioxidant capacity due to their ability to donate hydrogen and form stable radical intermediates. Non-structural phenolic compounds perform a variety of functions in plants, including acting as antioxidants.[97] Phenolic antioxidants are products of secondary metabolism in plants, and their antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting enzymes and scavenging free radicals. [98–100]

In this assay, the amounts of total phenolic content in the extracts was determined spectrophotometrically using Folin-Ciocalteu reagent and calculated as GAE. The Folin-Ciocalteu assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid

complexes, which are determined spectroscopically at 765 nm.

Results, as shown in **Table II**, indicate an increasing tendency of the phenolic content related to the extract solvent's polarity, while for most extracts, mid-polar solvents exhibit higher phenolic content. Lavender and Tilia extracts had significant increase of TPC related to the increased polarity of the solvent of 74,67% and 89.90%, respectively. On the contrary, although exhibiting higher average rates of TPC related to the other herbs, Sage and Sideritis rates of TPC related to the increased solvent's polarity, was reduced in a minimum level for Sage, however dramatically for Sideritis (over 60% reduction).

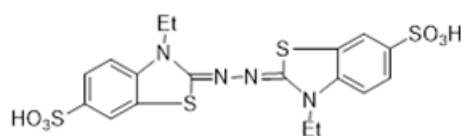
Regarding the preparation method, decoction samples had higher average results of phenolic content for 3 out of 4 herbs tested, while in the case of Sideritis, decoction samples showed a lesser reduction with regards to the solvent's increasing polarity.

Table II: Total Phenolic Content (mg/l) Pivot Table

	SOLVENTS	PE	DE	EA	BuOH	H ₂ O
Total Phenolic Content (mg/l)	Lavender Infusion	35.232	55.224	90.739	104.073	106.073
	Lavender Decoction	22.768	72.333	99.289	105.130	130.594
	Sideritis Infusion	99.127	44.233	46.722	33.763	38.874
	Sideritis Decoction	46.861	51.426	29.574	37.066	28.553
	Tilia Infusion	4.436	105.089	280.696	142.752	56.205
	Tilia Decoction	7.193	228.626	277.444	83.626	58.478
	Sage Infusion	46.378	83.255	173.118	184.489	63.732
	Sage Decoction	79.647	134.772	148.217	194.896	60.647

3.3 Assay of radical cation scavenging activity

The radical cation of ABTS is used for classification of relative inhibition capacity of flavonoids and phenolic compounds through their ability as electron or proton donors.



Scheme 2: Structure of f 2,2'-azinobis (3- ethylbenzothiazoline-6-sulfonicacid) (ABTS⁺)

When the radical interacts with antioxidants its concentration is reduced, recorded as absorption decrease. Thus, the higher the antioxidant activity of the tested substances the lower the absorption is since antioxidants interact with the colored cation in proportion to their concentration in each sample. The absorption maxima (λ_{max}) of $ABTS^+$ adopted by most investigators to spectrophotometrically monitored the reaction between the antioxidant and ABTS are 415 nm and

734 nm.

In this assay, ABTS is oxidized to its radical cation, $ABTS^+$ (**Scheme 2**), which is intensely colored. Antioxidant capacity is measured as the ability of test compounds to decrease the color reacting directly with the $ABTS^+$ radical. The results are expressed as Trolox equivalents and the measurements were taken after 6 minutes at 734 nm.

Table III: Antioxidant capacity of herbs' infusion and decoction preparation, via ABTS method (μM Trolox)

	SOLVENT	PE	DE	EA	BuOH	H ₂ O
Antioxidant capacity (μM Trolox)	Lavender Infusion	317.148	666.517	778.396	870.179	719.365
	Lavender Decoction	304.008	769.673	951.600	801.766	752.439
	Sideritis Infusion	129.027	346.062	858.623	780.610	466.019
	Sideritis Decoction	152.900	250.566	917.089	608.426	283.022
	Tilia Infusion	176.200	3777.17	8286.51	6,422.07	1346.65
	Tilia Decoction	303.280	4480.24	8118.59	2797.59	2148.59
	Sage Infusion	1074.72	2459.43	3440.95	7126.08	1046.47
	Sage Decoction	3496.67	33632.08	3786.13	30691,96	2752.89

The results (**Table III**) exhibit higher rates on extracts of mid-polar solvents in most cases regardless of the herb or the preparation method, while on the preparation method, the findings show that decoctions of each beverage presented higher average rates in 3 out of 4 cases and an overall rate of 42% higher than infusions. Out of a total of 40 samples, only 10 were above the average rates in this assay, 5 in the Sage group and 5 in the Tilia group, while 6 were decoctions and 4 infusions. Overall 10 lowest rates were observed in Lavender, Sideritis and Tilia in a distribution 2:6:2, while 6 of these rates were observed in Petroleum ether (PE) extracts, 2 in Diethyl ether (DE) extracts and only 2 in water extracts.

3.4 Interaction with Stable Free Radical of DPPH

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy inhibiting

the oxidative mechanisms that lead to degenerative diseases.

The DPPH assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. Several protocols are known considering DPPH assay; using different conditions such as different reaction times, solvents, pH taking different antioxidant standards.[21,82,101] The protocol followed in the present study, includes Trolox as the reference standard and two times of measurement of the extracts (20 minutes and 60 minutes).

According to our results, all the extracts interacted with the stable free radical DPPH and in most cases the reaction was over 50% regardless of the time of the measurement (20 minutes or 60 minutes), the preparation method or the solvent of the extract, as shown in **Figure 1** and **Figure 2**. In the measure-

ment of 20 minutes, only 17 samples rated less than 85% interaction, while during the 60 minutes measurement only one sample managed to pass the threshold of 50%. Among the lowest rates (15 samples with less than 50% reaction), 10 were observed in in Petro-

leum ether (PE) extracts, 4 of which were decoctions. Over the 60 minutes measurements, the overall reaction rate was 78.07%, with only 11 samples rating lower than average. None of the samples in the Sideritis group exhibited reaction less than 65%.

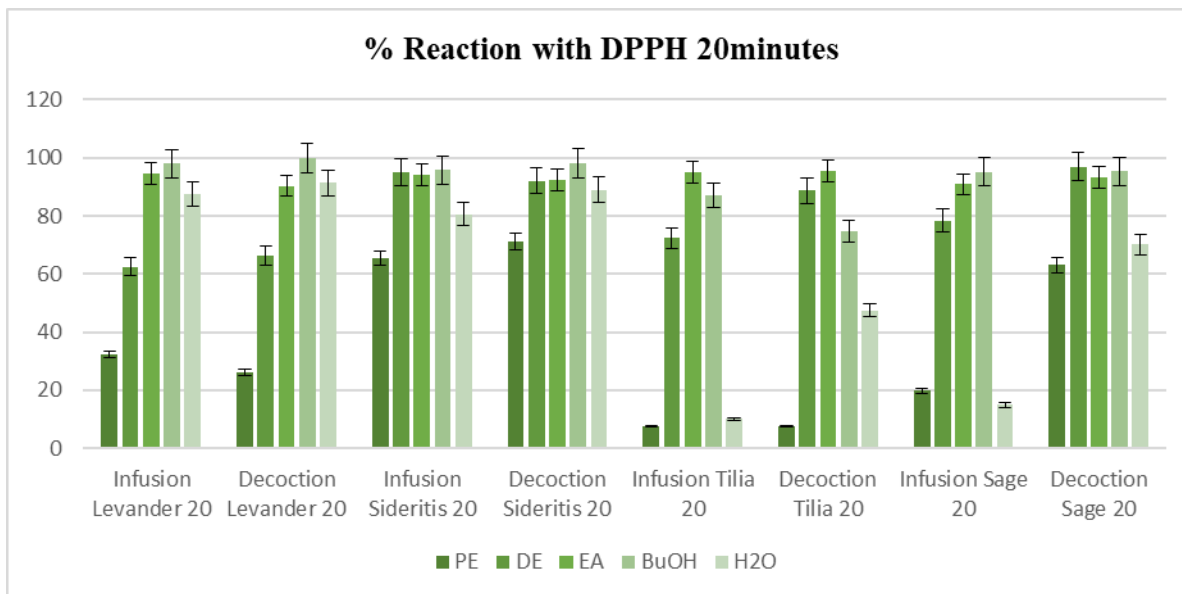


Figure 1: % Reaction of the extracts with DPPH standard radical at 20 minutes time. Each value represents the mean of three independent measurements in each sample (The results were averaged, and the standard deviation of absorbance was less than 10% of the mean). Statistical studies were done with student's *T*-test, $p < 0.05$

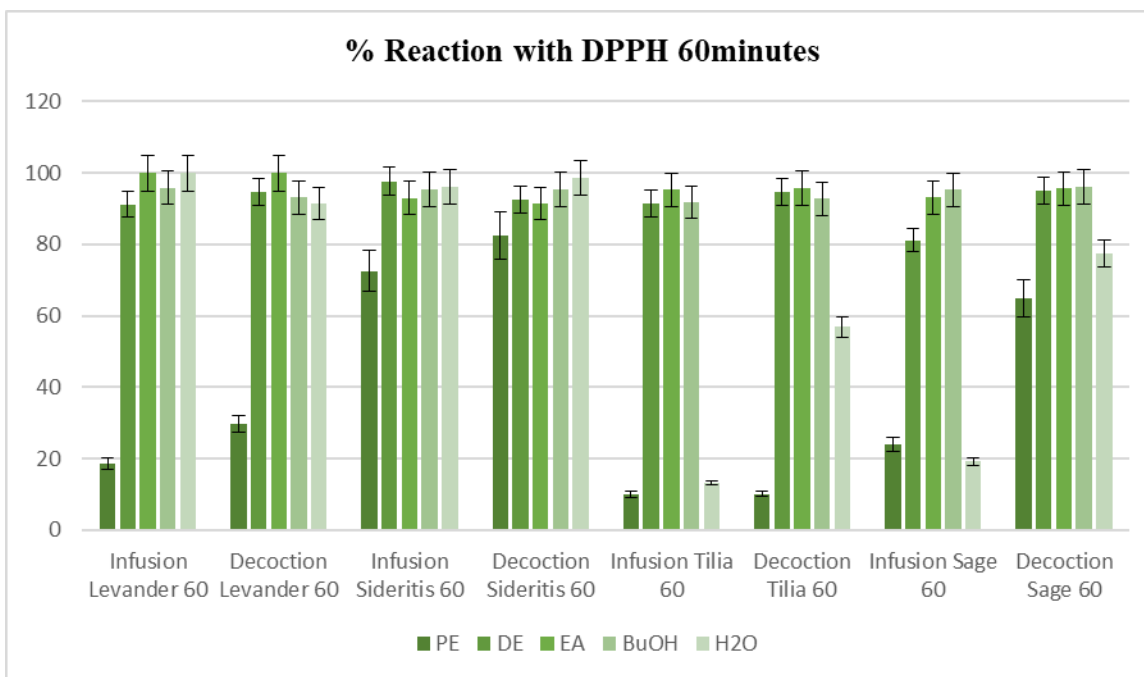


Figure 2: % Reaction of the extracts with DPPH standard radical at 60 minutes time. Each value represents the mean of three independent measurements in each sample (The results were averaged, and the standard deviation of absorbance was less than 10% of the mean). Statistical studies were done with student's *T*-test, $p < 0.05$

Considering the polarity of the solvents used, polar extracts show higher free radical scavenging activity than non-polar ones and this can be related to the presence of phenolic acids and flavonoids. The average reaction in the water extracts of all samples was 65.2% and Petroleum ether (PE) extracts averaged at 37.8%, while the average rate of the solvents in between was 91.7%.

3.5. Competition of the tested extracts with DMSO for hydroxyl radicals

Oxidative stress—the production and accumulation of reduced oxygen intermediates such as superoxide radi-

icals, singlet oxygen, hydrogen peroxide, and hydroxyl radicals—can damage lipids, proteins, and DNA.[102] The hydroxyl radical (OH) is one of the most powerful oxidizing agents, able to react unselectively and instantaneously with the surrounding chemicals, including organic pollutants and inhibitors.[103] Hydroxyl radicals are among the most reactive oxygen species and are considered to be responsible for some of the tissue damage occurring in inflammation. DMSO is a compound, which readily reacts with hydroxyl radical and it strongly suppresses hydrogen peroxide.[98,104]

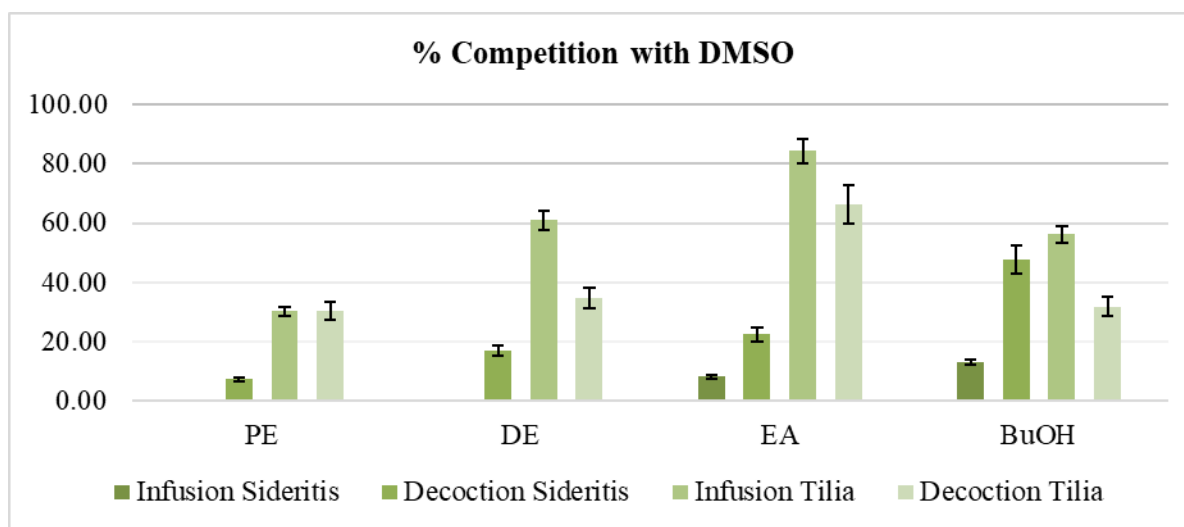


Figure 3: Competition of the tested extracts of Sideritis and Tilia beverages (Infusion and Decoction Preparation) with DMSO for hydroxyl radicals. Each value represents the mean of three independent measurements in each sample (The results were averaged, and the standard deviation of absorbance was less than 10% of the mean). Statistical studies were done with student's *T*-test, $p < 0.05$

Regarding the hydroxyl scavenging ability of the tested extracts from Sideritis - (Figure 3) an increasing tendency was observed depending on the solvent polarity. However not any pick was recorded in the aqueous samples. Similar increasing tendency occurs in Tilia samples. Overall, only 4 samples resulted with average rates higher than 50%, all of which were in the Tilia group, 3 of which were infusions.

3.6. % Inhibition of Linoleic Acid Lipid Peroxidation

AAPH was used as a free radical initiator to follow

oxidative changes of linoleic acid to conjugated diene hydroperoxide. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies *in vitro*. The water-soluble azo compound AAPH has been used as a clean and controllable source of thermally produced alkyl peroxy free radicals. In the AAPH assay, the highly reactive alkyl peroxy radicals are intercepted mainly by HAT from the antioxidant.[101,105,106]

In this experiment, the ability of Sage and Tilia extracts to inhibit the peroxidation of linoleic acid was in question. These two herbs have been previously tested for their antioxidant ability separately or in

contrast to each other and there are literature findings supporting non-significant differences in their capacity. [54–56]

Having found differences between them, further analysis of the antioxidant capacity as shown in the previous experiments further study was suggested.

The samples of both beverages were tested at two different times, one as concentrated sample (**Table V** (Sage) and (Tilia)) and then as a diluted sample (1:10 dilution) in order to estimate their inhibiting ability in a lower concentration. All samples presented inhibition with an average of 75% for the infusion treatment of preparation and 83% for the decoction.

Table IV 4: % Inhibition of lipid peroxidation of linoleic acid by Sage and Tilia beverage (Infusion and Decoction Preparation)

	PE	DE	EA	BuOH	H ₂ O
Infusion Sage	45 ± 3.5	85.6 ± 6.1	100 ± 9.1	100 ± 7.8	45 ± 3.9
Decoction Sage	95.8 ± 7.7	88.5 ± 8.2	92.3 ± 6.3	100 ± 8.2	41.6 ± 3.8
Infusion Tilia	39.6 ± 4.1	89.8 ± 8.5	82.8 ± 7.5	74.5 ± 6.3	8.4 ± 7.1
Decoction Tilia	26.7 ± 2.9	100.0 ± 8.8	96.1 ± 5.2	51.2 ± 4.1	6.5 ± 0.8

* Each value represents the mean of three independent measurements in each sample (The results were averaged, and the standard deviation of absorbance was less than 10% of the mean). Statistical studies were done with student's *T*-test, $p < 0.05$

Regarding the diluted samples (**Table V**), a rapid reduction of the % inhibition ability was observed in both herbs extracts. An average of 47.6% for the infusion of Sage and 61.6% for the decoction was estimated. Tilia diluted samples had an average decrease of 41.6% for the infusion and 31.1 % for the decoction treatment compared to the concentrated samples.

Table V: % Inhibition of lipid peroxidation of linoleic acid by Sage and Tilia beverage (Infusion and Decoction Preparation Diluted Samples 1:10)

	PE	DE	EA	BuOH	H ₂ O
Infusion Sage	87.1 ± 7.6	35.5 ± 3.3	46.6 ± 4.2	54.4 ± 4.2	3.5 ± 0.4
Decoction Sage	25.6 ± 2.9	30.9 ± 2.2	28.7 ± 2.1	24.6 ± 2.1	0.4 ± 0.0
Infusion Tilia	13.8 ± 0.8	23.2 ± 1.9	18.4 ± 1.6	25.1 ± 2.1	6.6 ± 0.6
Decoction Tilia	23.7 ± 2.5	26.4 ± 1.8	40.5 ± 3.9	27.8 ± 2.6	6.7 ± 0.4

* Each value represents the mean of three independent measurements in each sample (The results were averaged, and the standard deviation of absorbance was less than 10% of the mean). Statistical studies were done with student's *T*-test, $p < 0.05$

The overall average decreased from 68.47% (original samples) to 27.47% (diluted samples). Among the original samples, 12 were above average rates, 11 of which were observed in mid-polarity solvents, while among the diluted samples, only 8 were above average rates, 7 of which were observed in mid-polarity solvents.

3.7. Organoleptic Evaluation

The primary goal of the organoleptic evaluation was to get a feedback on whether a beverage of these herbs is acceptable for consumption with respect to the preparation method without adding any sweeteners.

There has been an evaluation of various characteristics such as turbidity, color, bitterness, acidity using a unipolar scale in a questionnaire (Results not shown) as well as flavor, taste and liking using a bipolar scale (**Scheme3**), it was considered important to present only the results of the liking of each beverage and discuss the rest of our findings in reference to other organoleptic characteristics.

EXTREMELY UNPLEASANT	VERY UNPLEASANT	MEDIUM UNPLEASANT	A LITTLE UNPLEASANT	NEITHER	A LITTLE PLEASANT	MEDIUM PLEASANT	VERY PLEASANT	EXTREMELY PLEASANT
-4	-3	-2	-1	0	+1	+2	+3	+4

Scheme 3: Bipolar Scale rating

Results of the sensory evaluation (**Figures 5 and 6**) did not show any extreme reactions of liking in any of the cases. In the case of the infusion samples, Lavender had the least acceptability with 46% of the participants describing the beverage as “Very Unpleasant” or “Extremely Unpleasant”. Similarly, the responses were Medium to Extremely Unpleasant for the Tilia and Sideritis samples at a rate of 36% and 35%, respectively. Finally, Sage samples, did not receive any “Very Unpleasant” or “Extremely Unpleasant” rates, however, 60% of the participants found the beverage moderately acceptable.

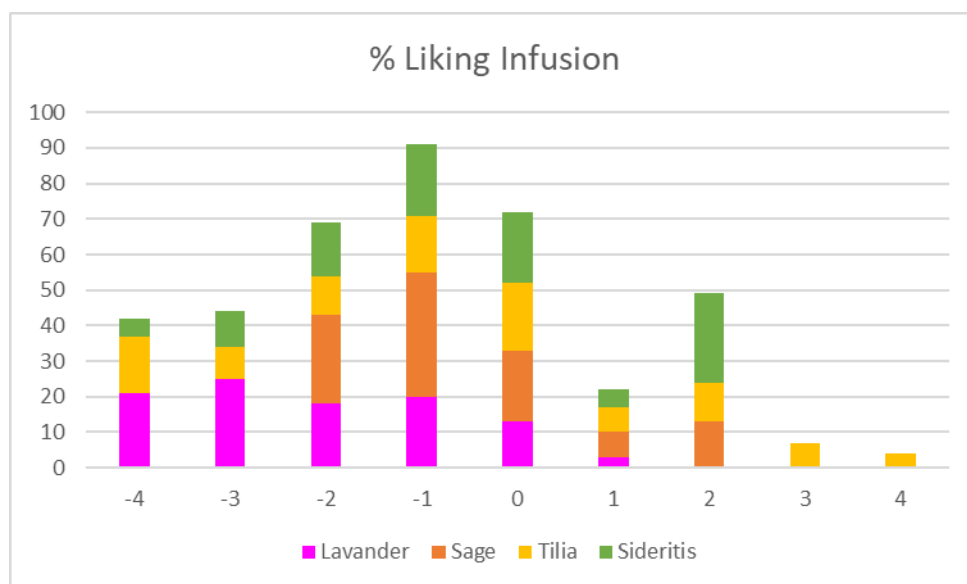


Figure 5: % Liking of Infusion treatment for all beverages (Pivot Chart)

The decoction samples were overall slightly better accepted. In this preparation method, Sage had no negative responses, Lavender's "Very Unpleasant" or "Extremely Unpleasant" rates were 40%, while the responses of Medium to Extremely Pleasant for the Tilia and Sideritis samples reflected 21% and 30% of the participants, respectively.

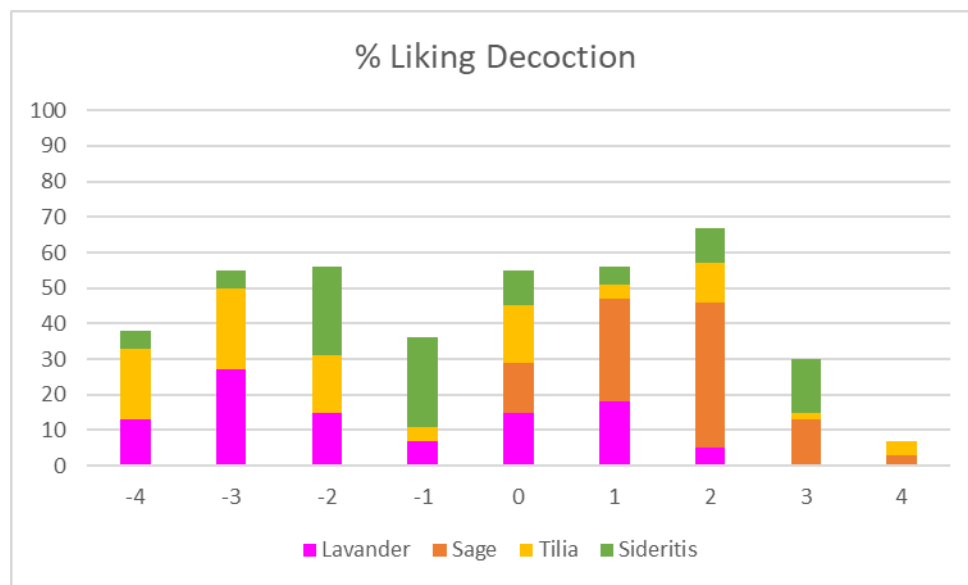


Figure 6: % Liking of Decoction treatment for all beverages (Pivot Chart)

DISCUSSION

Spices and herbs are rich sources of powerful antioxidants. Spices and herbs have been used for flavour, colour and aroma for more than 2000 years.[107] The Mediterranean diet is currently attracting interest because of its health benefits that may be due, in part, to the high content of this diet in antioxidant phytochemicals.[108]

Our study examined the antioxidant characteristics of four aromatic plants participating in the Mediterranean Diet as beverages. Results showed a strong relation between the antioxidant activity and the phenolic content of each beverage, regardless of the manner of preparation, as the total antioxidant activity, is increased with the content of phenols in most cases. This finding has been previously observed by a number of studies, involving different set of herbs and indicating the role of phytochemicals in this biological activity.[109] Different treatment of the samples appeared to minimally influence the concentration of phenolic compounds, as 3 out of 4 beverages presented higher phenolic content. Sage and Tilia demonstrated the highest rates of antioxidant activity in one

or both test (ABTS and/or DPPH), which in the case of Sage can be related to its consisting of flavone glycosides and a range of rosmarinic acid derivatives, as it has been already presented in previous studies.[110]

In all cases, decoction samples presented higher antioxidant results e.g. in the Sage beverage and in the Tilia beverage. Although these two herbs had the highest antioxidant activities, differences between Tilia and Sage beverages were not statistically significant, as it has been previously observed by a previous study.[56] However, further studies showed that Sage's beverage presented stronger inhibitory activity against linoleic acid peroxidation.

Tilia samples and specifically, those of the infusion treatment were found to be stronger scavengers for hydroxyl radicals compared to the samples of Sideritis, although a recent study indicated that linden flowers beverage extract could not be as important as diet-derived antioxidants in preventing oxidative damage in the tissues by reducing the lipid oxidation or inhibiting the production of ethanol-induced free radicals in rats.[111]

The decoction samples of Lavender beverage

presented medium results in relation to the phenolic content, which can be related to the fact that only the flowers of the plant were used, while literature supports that leafy stalks may present higher activity. [112] On the other hand, Sideritis showed better results in the infused samples regarding the phenolic content and the reaction with ABTS and DPPH radical, although at the decoction samples' scavenging ability was reported stronger.

In both cases (infusion and decoction), there has been intense and steep discoloration in the interaction with DPPH as well as in the interaction with the ABTS cationic radical. This observation was more significant in the case of the more condensed samples, and further experimental study of the action is required.

On the organoleptic evaluation all beverages received moderate reviews. with the main comment from the testers being that the beverage was missing a sweetener and that in lack of it, it would be unlikely to be consumed. Finally, beverages prepared as decoctions had slightly better reviews in reference to acceptability, while Sage and Tilia presented a better organoleptic profile.

CONCLUSIONS

This study presented a series of Mediterranean herbs that have been used for the preparation of beverages at house conditions. Although all tested beverages exhibited significant antioxidant activity, Sage was found to present the highest antioxidant activity in parallel to high sensory evaluation. Especially, Sage's beverage, prepared using decoction procedure seems to be the proposed beverage for a preparation at house conditions. There is a wide range of aspects that need to be tested in the future in order to provide a better understanding of the necessity of including aromatic plants' beverages in regular diet.

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