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Correlation of total polyphenolic content with antioxidant and antibacterial activity of 24 extracts from Greek domestic *Lamiaceae* species

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ABSTRACT

Lamiaceae family species are considered important because of their use in folk medicine, culinary and flavouring throughout the world. Their interesting bioactivities are attributed mainly to essential oils, polyphenols and terpenes. However, there are only few studies about polyphenolic extracts from *Lamiaceae* plants. Thus, 24 polyphenolic extracts from three *Lamiaceae* genera, *Salvia*, *Mentha* and *Sideritis*, collected in Greece were examined for antioxidant and antibacterial activity in correlation with their polyphenolic content. The results showed that the tested polyphenolic extracts had strong free radical scavenging activity against DPPH· and $ABTS^{\cdot+}$ radicals and protected from hydroxyl and peroxyl radical-induced DNA damage. Moreover, five extracts inhibited *Staphylococcus aureus* growth. Furthermore, the results showed that the total polyphenolic content is not correlated with the above activities, although this relation was different within each plant genus. This is the first study regarding the antioxidant and antibacterial activity of *Salvia pomifera* ssp. *calycina*, *S. pomifera* ssp. *pomifera*, *Mentha microphylla* and *Sideritis raeseri* ssp. *attica* species, and one of the few concerning protection from DNA damage and antibacterial activity of polyphenolic extracts from the rest of the tested species.

1. Introduction

The family of *Lamiaceae* consists of about 230 genera and 7100 species worldwide (Harley et al., 2004). Many species of the *Lamiaceae* family are considered of high importance because of their uses in medicine, culinary and cosmetics, and production of essential oils (Harley et al., 2004). Some of the major genera belonging to *Lamiaceae* family are *Salvia*, *Mentha* and *Sideritis*. The genus *Salvia* includes approximately 900 species that are cultivated throughout the world for use in folk medicine and for culinary purposes (Kamatou et al., 2008). Essential oils and extracts from *Salvia* species have been shown to possess antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, hypoglycaemic and anticarcinogenic properties (Kamatou et al., 2008; Tenore et al., 2011; Esmaeili and Sonboli, 2010). The genus *Mentha* comprises 20 species which are distributed all over the world (McKay and Blumberg, 2006). The aerial parts from *Mentha* species have been widely used for treatment of cold, cholera, bronchitis, tuberculosis, sinusitis and for their diuretic, carminative, antiflatulent, expectorant, antitussive, antioxidant and antimicrobial properties (McKay and Blumberg, 2006; Kamkar et al., 2010; Saleem et al., 2000). The genus *Sideritis* includes at least 150 species found mainly in the Mediterranean area (González-Burgos et al., 2011). *Sideritis* species have been used in folk medicine for their antimicrobial, antiulcerogenic, digestive and anti-inflammatory properties (González-Burgos et al., 2011; Köse et al., 2010). The aerial parts of plants from genus *Sideritis* are used in Greece as decoction, known as 'mountain tea', against especially gastrointestinal disorders and common colds (Aligiannis et al., 2001).

Extracts from many species of the *Lamiaceae* family exhibit important antioxidant potency, namely they are scavengers of reactive oxygen species (ROS) (Kamatou et al., 2008; McKay and

Abbreviations: AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl; GA, gallic acid; GAE, gallic acid equivalent; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase enzyme; MIC, minimum inhibitory concentration; Na_2CO_3, sodium carbonate; $OH^{\cdot}$, hydroxyl radical; $ROO^{\cdot}$, peroxyl radical; RSC, radical scavenging capacity; TPC, total polyphenol content.

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Blumberg, 2006; González-Burgos et al., 2011). ROS are constantly produced in cells by cellular metabolism and by exogenous agents. They are essential for life, because they are involved in cell signaling and are used by phagocytes for their bactericidal action (Halliwell and Gutteridge, 1999). However, ROS can react with various biomolecules in cells, as lipids, proteins and DNA and cause damage to these molecules (Halliwell and Gutteridge, 1999). Thus, an imbalance between production of ROS and antioxidant defenses, which is called oxidative stress, may lead to degenerative processes such as cancer, aging, cardiovascular and neurodegenerative diseases (Halliwell and Gutteridge, 1999). Especially, as regards DNA damage, ROS induce numerous lesions in DNA including deletions, base modifications, single- and double-strand breakages that could lead to the development of cancer (Halliwell and Gutteridge, 1999). Therefore, antioxidant agents are considered beneficial for human health.

Moreover, *Lamiaceae* species have been used widely for their anti-microbial properties. Micro-organisms are causative factors for the pathogenesis of various diseases as well as for the spoilage and deterioration of food, pharmaceutical and cosmetic products. Furthermore, in the last years, the number of bacteria resistant to current antibiotics has increased dramatically (Theuretzbacher, 2011), thus there is a great need for discovering new anti-microbial agents. Also, the mistrust of anti-microbial agents of synthetic origin due to their potential toxicity and carcinogenicity (Anwar-Mohamed and El-Kadi, 2007; Tsay et al., 2007) has intensified the efforts for discovering natural alternatives.

Essential oil, polyphenols and terpenes are considered the main chemical compounds responsible for the pharmacological activity of species belonging to *Salvia*, *Mentha* and *Sideritis* genera (González-Burgos et al., 2011; McKay and Blumberg, 2006; Kamatou et al., 2008). However, most studies have focused on essential oils, while there are much fewer reports regarding bioactivities of polyphenolic extracts from these genera. Therefore, the aim of the present study was to examine the antioxidant and antibacterial activities of polyphenolic extracts from six *Salvia* species (*Salvia pomifera* ssp. *calycina, S. pomifera* ssp. *pomifera, S. fruticosa, S. sclarea, S. argentea* and *S. officinalis*), four *Mentha* species (*Mentha microphylla, M. longifolia, M. pulegium* and *M. aquatic*) and two *Sideritis* species (*Sideritis raeseri* ssp. *raeseri* and *S. raeseri* ssp. *attica*) collected in Greece. Extracts were obtained using water and methanol (or ethanol), since polar polyphenols tend to be more soluble in water and non-polar ones are more soluble in non-aqueous solvents. Thus, in total, twenty-four extracts were tested. For evaluating the antioxidant capacity of the extracts, DPPH and ABTS free radical scavenging assays were used. Also, the potential protective activity of the extracts against DNA damage induced by hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals was examined. Hydroxyl radicals can be produced inside cells by various reactions as Fenton and UV-induced decomposition of H_2O_2 (Halliwell and Gutteridge, 1999). Peroxyl radicals are formed in a variety of physiological and pathological processes after abstractions of hydrogen atoms by $OH^{\cdot}$ followed by the addition of O_2 to the resulting carbon-centred radicals (Dix and Aikens, 1993). For example, when a free radical within or on the outside of a cell attacks the fatty acids of the cell membrane structures, then $ROO^{\cdot}$ radicals are produced comprising a major initiating factor of lipid peroxidation chain reactions that may result to cell membrane collapse (Halliwell and Gutteridge, 1999). Moreover, $ROO^{\cdot}$ can oxidize DNA bases to their hydroxyl derivatives leading subsequently to various pathological conditions (Simandan et al., 1998). Moreover, the antibacterial activity of extracts was investigated against Gram-positive (i.e. *Staphylococcus aureus*) and Gram-negative (i.e. *Pseudomonas aeruginosa*) bacteria. Finally, all these activities were correlated with the polyphenolic content of the extracts in order to examine if plant polyphenols are responsible for them. Specifically, this is the first report concerning the antioxidant and antibacterial activity of *S. pomifera* ssp. *calycina, S. pomifera* ssp. *pomifera, M. microphylla* and *S. raeseri* ssp. *attica* species. As regards the other species, there are a number of studies especially on the antioxidant activity but only few, if any, concerning protection of polyphenolic extracts from DNA damage and antimicrobial activity.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Folin–Ciocalteu reagent, sodium carbonate, gallic acid, methanol, horseradish peroxidase enzyme (HRP), hydrogen peroxide, ethidium bromide, tetracycline, 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) and agarose were purchased from Sigma–Aldrich (Steinheim, Germany). Mueller–Hinton broth and agar were purchased from CODA (Spain), tetracycline was purchased from SERVA (Heidelberg, Germany) and pBluescript-SK + plasmid DNA was obtained from Stratagene (CA, USA).

2.2. Plant materials

Plant material was collected by Dr. E. Kalpoutzakis from several areas of the Greek province. Aerial parts (herba in flowering stage) of six *Salvia*, four *Mentha* and two *Sideritis* species were collected in the period of May–July 2007. In more details, *S. fruticosa* was obtained from the village Zaros (Heraklion, Crete island), *S. sclarea* and *S. officinalis* from the area 'Papigo' (Ioannina, N.W. Greece), *S. argentea* from the Mt. Parnassos (C. Greece), *S. pomifera* ssp. *pomifera* from the area Topolia (Chania, Crete island), *S. pomifera* ssp. calycina from the Mt. Parnonas (Arkadia, S. Greece). Additionally, *M. microphylla*, *M. longifolia* and *M. pulegium* were obtained from the village Zaros (Heraklion, Crete island), and *M. aquatica* was collected from Arkadia (S. Greece). Finally, *S. raeseri* ssp. *raeseri* was collected from Mt. Agrafa (W. Greece) and *S. raeseri* ssp. *attica* came from Mt. Parnis (C. Greece).

Methanolic and aqueous extracts of the pulverized air-dried aerial parts of the above mentioned *Lamiaceae* species were obtained using an accelerated solvent extractor (ASE 300, DIONEX Co.). In more details, the sample (~20 g) was loaded into the stainless steel extraction cell, filled with solvents for about 1 min, and pressurized for about 5 min; After a pressure of 105 bar was obtained, heating was commenced for 15 min (static time) whilst the pressure was maintained at 90–130 bar. Extraction temperatures of 50 and 70 °C were assigned for methanol and aqueous extracts, respectively. The unit could be flushed with fresh solvent (60 ml) pumped through the sample and entire pathway within 60 s. Purging was conducted for 1.5 min between extractions, often with nitrogen gas. The extraction for each solvent was repeated three times. The final extracts were collected in clear glass vials (250 ml). The total extraction time consumed was 60 min for each extract.

2.3. Estimation of total polyphenolic content

The phenolic content of the extracts was determined by Folin–Ciocalteu colorimetric method (Singleton et al., 1999). Plant extracts (0.1 mL at proper dilution) were added to 5 mL of deionized water and 0.5 mL of Folin–Ciocalteu reagent. In the blank samples, 5.1 mL of deionized water and 0.5 mL of Folin–Ciocalteu reagent were added. After mixing, the samples were left for 3 min at room temperature and 1.4 mL of a 25% w/v solution of sodium carbonate (Na_2CO_3) and 3 mL of deionized water were added. The mixture was left for 1 h at room temperature in the dark and the absorbance at 765 nm was measured using a Hitachi U-1500 spectrophotometer. Optical density of the extracts alone (0.1 mL at proper dilution) in 1.4 mL of a 25% w/v solution of Na_2CO_3 and 8.5 mL of deionized water was also measured at 765 nm. The total phenolic content was determined by a standard curve of absorbance values derived from standard concentration solutions of gallic acid (GA). The total polyphenol content (TPC) was expressed as mg of gallic acid equivalent per g of dried extract (mg GAE/gr dry weight). Each sample was tested in triplicate.

2.4. Free radical-scavenging ability by the use of a stable DPPH radical

The DPPH radical scavenging activity of plant extracts was evaluated as described previously (Spanou et al., 2007). Briefly, 1.0 mL of freshly made methanolic solution of DPPH radical ($DPPH^{\cdot}$) (100 μM) was mixed with tested extract solution at different concentrations (5–100 μg/mL). The contents were vigorously mixed, incubated at room temperature in the dark for 20 min and the absorbance was read at 517 nm. In each experiment, the tested extract alone in methanol was used as blank and $DPPH^{\cdot}$ alone in methanol was used as control. The percentage of radical scavenging capacity (RSC) of the tested extracts was calculated according to the following equation: $RSC\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$ where $A_{control}$ and A_{sample} are the absorbance values of the control and the test sample respectively. Moreover, in order to compare the radical scavenging efficiency of the extracts, IC_{50} value