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Research Paper

Chemical composition and biological activities of extracts from three *Salvia* species: *S. blepharochlaena*, *S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca*

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ABSTRACT

The genus *Salvia* has recently attracted great attention due to its notable biological activities. Within this context, in this study, the chemical characterization and biological effects of three extracts (dichloromethane (DCM), methanol (MeOH), and water) from three *Salvia* species (*S. blepharochlaena* (SB), *S. euphratica* var. *leiocalycina* (SE), and *S. verticillata subsp. amasica* (SV)) were assessed. For the chemical characterization, the qualitative and quantitative analysis of phenolic components in the methanol extracts was carried out by high-performance liquid chromatography with electrospray ionization mass spectrometry detection (HPLC-UV-ESI-MS^n). Total phenolic, flavonoid and phenolic acid contents were also studied. Concerning the biological effects, antioxidant (DPPH and ABTS free radical scavenging; ferric (FRAP) and cupric (CUPRAC) reducing power; phosphomolybdenum, and metal chelating assays), enzyme inhibitory (cholinesterase, tyrosinase, amylase, glucosidase, lipase, and elastase) and cytotoxic effects (A-549 and MCF-7 cell lines) were evaluated. After the evaluation of the phytochemical profile by HPLC-UV-ESI-MS^n, it was observed that the main compound in the analyzed extracts was rosmarinic acid, which was present at high concentrations, particularly in SV, which presented rosmarinic acid levels higher than the usual levels found in other *Salvia* species or related plants. Generally, the SV-water extract presented the strongest antioxidant abilities with higher levels of total bioactive compounds. However, the studied DCM extracts had higher enzyme inhibitory potentials compared with MeOH and water extracts. SE-DCM exerted the most potent cytotoxic effects, followed by SB-water and SB-MeOH extracts.

1. Introduction

In the last few years, natural products are gaining special interest in different scientific areas. Medicinal plants are rich sources of biologically-active compounds such as phenolics, alkaloids, and terpenes. The last scientific studies showed that these bioactive compounds are responsible for multi-functional biological effects including antioxidant, anticancer, antimicrobial, and anti-inflammatory (Sen and Samanta, 2014). Considering the above mentioned, many plant matrices are considered as promising raw materials for the design of novel products in the pharmaceutical, cosmetic, and food industries. It is thus important to carry out new studies in this area, in which uninvestigated plants could represent important sources of bioactive compounds in order to develop products with new industrial applications.

The prevalence of some diseases (Alzheimer's disease (AD), diabetes mellitus (DM) and obesity) has been increasing in recent decades in most regions of the world. For example, 422 million adults were affected with DM in 2014 and the prevalence has nearly doubled since 1980 (WHO, 2016). In addition, the global prevalence of obesity was 5% for men and 8% for women in 1980. In 2008, the prevalence was 10% for men and 14% for women (Bischoff et al., 2016; WHO, 2017). Within this framework, these diseases can be considered as "global health problems". As a result, novel, safe, and effective therapeutic approaches are needed to control these diseases. Key enzyme inhibition

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theory is proposed as one of the most effective approaches for the management of these diseases. According to this theory, the inhibition of key enzymes could alleviate the symptoms present in these diseases. In this direction, cholinesterase for AD, amylase/glucosidase for DM, lipase for obesity, tyrosinase for skin disorders, and elastase for aging, are useful therapeutic targets. Several enzyme inhibitors have been chemically produced. However, most of these synthetic inhibitors have severe side effects such as gastrointestinal disturbances and toxicity (Birari and Bhutani, 2007; Dey et al., 2016; Etxeberria et al., 2012; Lee et al., 2016). In this context, many phytochemicals and natural compounds have been studied and reported as promising enzyme inhibitors in recent studies (Abbas et al., 2016; Bello et al., 2017; Lee et al., 2016; Rauf and Jehan, 2017).

Lamiaceae family is one of the most investigated plant families, many of its representatives being used as sustainable sources of functional products (Sacchetti et al., 2004). Within this family, the genus *Salvia* has gained much attention due to its biological properties, in a similar way to *Thymus, Origanum* or *Sideritis*. The genus *Salvia* is represented by more than 900 species in the world (Walker et al., 2004). In Turkey, the genus comprises about 95 species, 50% of which are endemic (Tan et al., 2017). The members of this genus are known as “adaçayı” in Turkish (Bulut et al., 2011; Tetik et al., 2013). The *Salvia* genus has many traditional usages, mainly as herbal teas, appetizers, as well as used to alleviate gastrointestinal problems, colds, and abdominal pains (Baytop, 1999; Bulut et al., 2011; Gürdal and Kültür, 2013; Lopresti, 2017; Ugulu et al., 2009; Wu et al., 2012). In addition, there is a vast amount of literature describing biological activities of the members of this genus, including antioxidant, antimicrobial, antiviral, enzyme inhibitory, and anticancer (Alimpić et al., 2017; Bahadori et al., 2017a, 2017b, 2015; Civra et al., 2017; Flores-Bocanegra et al., 2017; Tan et al., 2017; Wang et al., 2017b). However, there is little information on the chemical profiles and biological activities of *S. blepharochlaena, S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca* (Janicsák et al., 2011; Kunduhoglu et al., 2011; Orhan et al., 2013; Ulubelen et al., 2001).

In this sense, the aim of this study is to shine new lights on the biological effects and chemical characterization of the above-mentioned *Salvia* species. Phytochemical profiles and quantification of phenolics in methanol extracts were accomplished by HPLC-UV-ESI-MS^n. For biological properties, antioxidant (free radical scavenging, reducing power, total antioxidant capacity – by phosphomolybdenum method – and metal chelating), enzyme inhibitory (against cholinesterase, tyrosinase, amylase, glucosidase, lipase, and elastase) and cytotoxic effects (by A-549 and MCF-7 cell lines) of three extracts (DCM, MeOH and water) of *S. blepharochlaena* (SB) *S. euphratica* var. *leiocalycina* (SE) and *S. verticillata* subsp. *amasiaca* (SV) were tested. The obtained results can provide new and valuable perspectives on the genus and stand as a bridge between past and future studies, leading to the development of novel products for the phyto-pharmaceutical industry as well as for agro-based products.

2. Materials and methods

2.1. Plant materials and preparation of extracts

The aerial parts of three *Salvia* species were collected in the east region of Turkey and the locations are given below. The plant's identification was performed by botanist Dr. Murad Aydin Sanda. Voucher specimens of the studied *Salvia* species were deposited at KNYA herbarium of Selcuk University.

1. *Salvia blepharochlaena* Hedge & Hub. Mor.: Between Avanos and Kayseri, 38°42'37“N, 34°53'52“E, 992 m.
2. *Salvia euphratica* Montbret & Aucher. var. *leiocalycina* (Rech.) Hedge.: Sivas, Gürün, Şugul Valley, 38°44'20“N, 37°14'31“E, 1367 m.
3. *Salvia verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm.: Sivas, between Gürün and Gökpınar, 38°50'53“N, 37°07'04“E, 1389 m.

The aerial parts of the selected species were analyzed. First, they were dried in the dark at room temperature. Then, they were ground to powder using a laboratory mill. The samples (10 g) were stirred with DCM or MeOH at room temperature for 24 h. Then, the extracts were concentrated to dryness using a rotary evaporator. As for water extracts, 5 g of plant material was boiled in 100 mL water for 30 min. Then, the extracts were lyophilized. The obtained extracts were stored in dark glass at 4 °C until further analyses.

2.2. Chromatographic conditions

The analysis of the phenolic composition of the studied species was carried out by HPLC-UV-MS^n. 5 mg of MeOH extracts were re-dissolved in 1 mL MeOH. After filtration through 0.45 µm PTFE membrane filters, 10 µL of solution was injected using a flow rate of 0.4 mL/min.

The HPLC system used was an Agilent Series 1100, which had an autosampler, a vacuum degasser, and a binary pump. For UV measurements, a G1315 B diode array detector (Agilent) was used. The HPLC column was a Luna Omega Polar C_{18} analytical column of 150 × 3.0 mm and 5 µm particle size (Phenomenex, Madrid, Spain). A Polar C_{18} Security Guard cartridge of 4 × 3.0 mm (Phenomenex) was also used. The following gradient mode, using CH_3CN and water-formic acid (100:0.1, v/v), was used: (a) initial mobile phase, 10% CH_3CN; (b) linear increase from 10% to 25% CH_3CN (0–25 min); (c) 25% CH_3CN (25–30 min); (d) linear increase from 25% to 50% CH_3CN (30–40 min); (e) linear increase from 50% to 100% CH_3CN (40–42 min); (f) 100% CH_3CN (42–47 min). Then, CH_3CN percentage was returned to 10%, allowing a 7 min stabilization time before the next sample injection.

For MS analysis, an ion trap mass spectrometer with a negative ion mode (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) was used. The analytical conditions for MS analysis were given in our previous paper (Llorent-Martínez et al., 2017).

2.3. Total phenolics, flavonoids, and phenolic acids

The total bioactive compounds (phenolics and flavonoids content) of the studied *Salvia* extracts were obtained using Folin-Ciocalteu and $AlCl_3$ methods, respectively. The experimental procedures were reported in our previous paper (Slinkard and Singleton, 1977; Zengin et al., 2016). The contents were expressed as gallic (mg GAEs/g extract) acid and rutin equivalents (mg REs/g extract), respectively. The amount of total phenolic acids was expressed as caffeic acid equivalents as reported by Vladimir-Knežević et al. (2011).

2.4. Biological activities evaluation

Antioxidant potentials, enzyme inhibitory effects, and cytotoxic properties were evaluated for the biological activities of the studied *Salvia* species. Among the used antioxidant methods were included: free radical scavenging (ABTS and DPPH); ferric (FRAP) and cupric reducing power (CUPRAC); phosphomolybdenum, and metal chelating assays. The antioxidative potentials were expressed as trolox equivalents (EDTA was only used for metal chelating assays). Anti-cholinesterase, anti-tyrosinase, anti-amylase, anti-glucosidase, anti-lipase, and anti-elastase assays were tested for detecting enzyme inhibitory effects. The enzyme inhibitory effects were evaluated as standard compound equivalents. Briefly, galantamine was used for AChE and BChE, kojic acid was used for tyrosinase, and orlistat was used as standard for lipase, while acarbose was used for α-amylase and α-glucosidase; catechin was used as standard for elastase inhibition assay. The extracts were evaluated at 0.5–2 mg/mL in the antioxidant and enzyme inhibitory assays. All experimental procedures were assessed as reported by Grochowski et al. (2017) and Chlapanidas et al. (2013).