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Composition and biological effects of *Salvia ringens* (Lamiaceae) essential oil and extracts

Ana Alimpić[a,*], Dejan Pljevljakušić[b], Katarina Šavikin[b], Aleksandar Knežević[a], Milena Ćurčić[c], Dragan Veličković[d], Tatjana Stević[b], Goran Petrović[e], Vlado Matevski[f], Jelena Vukojević[a], Snežana Marković[c], Petar D. Marin[a], Sonja Duletić-Laušević[a]

[a] *Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia*
[b] *Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia*
[c] *Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34 000 Kragujevac, Serbia*
[d] *College of Agriculture and Food Technology, Ćirila and Metodija 1, 18400 Prokuplje, Serbia*
[e] *Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
[f] *Institute of Biology, Faculty of Natural Sciences and Mathematics, University "Ss. Cyril and Methodius" and Macedonian Academy of Sciences and Arts, Blvd. Goce Delcev 9, 1000 Skopje, Macedonia*

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ABSTRACT

This comprehensive study was carried out in order to investigate composition and biological activities of essential oil and extracts of *Salvia ringens* Sibth. & Sm. (Lamiaceae) originating from Macedonia. Major components of the oil, analyzed using GC-FID and GC–MS, were monoterpenes 1.8-cineole (31.99%), camphene (17.06%), borneol (11.94%) and α-pinene (11.52%). HPLC analysis showed presence of 17 phenolic components, mainly in methanol and ethyl acetate, followed by ethanol, water and dichloromethane extracts. Total phenolics and flavonoids as well as DPPH, ABTS, and FRAP activities were measured spectrophotometrically. Essential oil, ethanol, and water extracts showed antimicrobial activity using microdilution method. Ethanol and water extracts performed cytotoxic activity against colon carcinoma HCT-116 cell line using MTT assay. According to the obtained results, *S. ringens* herb can be considered as the potential source of the essential oil and/or raw material for the extraction and isolation of natural compounds with a range of biological activities.

1. Introduction

The Lamiaceae family comprises aromatic plants widely used as spices and medicinal plants, such as rosemary, basil, sage, lavender, thyme, mint, and oregano. The flavor of herbs and spices derives from essential oil components which make food more pleasant and, at the same time, show a wide spectrum of biological activities (Miguel, 2010). Some of the Lamiaceae species were reported as a rich source of phenolic compounds possessing strong antioxidant activity, and therefore, can be applied in prevention and therapy of free-radical associated diseases such as atherosclerosis, cancer, cardio-vascular disease, immune-system decline, brain dysfunction, cataracts, skin diseases (Asadi et al., 2010; Kamatou et al., 2010; Li et al., 2008) and may also serve as natural food preservatives (Miguel, 2010).

The genus *Salvia* is the largest member of the family Lamiaceae which comprises about 1000 worldwide distributed species. In Flora of Europe, the genus is represented by 36 species grouped into 7 sections (Hedge, 1972). *In vitro* pharmacological investigations showed its antioxidant, antibacterial, antifungal, antiviral, cytotoxic, neuroprotective, antiinflammatory, and tumorigenesis-preventing as well as ecological significance such as pest-toxic and repellent and other activities (Asadi et al., 2010; Baričević and Bartol, 2000; Ben Farhat et al., 2009; Kamatou et al., 2010; Orhan et al., 2012; Veličković et al., 2002). Aerial parts of these plants usually contain flavonoids and triterpenoids as well as essential oils with volatile compounds such as monoterpenoids, while diterpenoids are the main compounds in roots (Baričević and Bartol, 2000). It is a rich source of polyphenols, with an excess of 160 polyphenols having been identified, some of which are unique to the genus (Lu and Foo, 2002).

Salvia ringens Sibth. & Sm. is a hardy herbaceous perennial herb, heights of up to 60 cm. Specific epithet, *ringens*, refers to the wide open two-lipped flowers. It inhabits dry stony and grass-covered

* Corresponding author. Fax: +381 113246655.
E-mail address: alimpic.ana@bio.bg.ac.rs (A. Alimpić).

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places of South and Eastern parts of Balkan Peninsula, just extending to southeast Romania (Hedge, 1972). This is drought tolerant and long lived and highly valued as ornamental and melliferous plant species due to ponderous leaf rosette, attractive purple flowers, and pleasant intense fragrance.

Previous researchers have partially investigated composition and biological activities of *S. ringens* essential oil and/or extracts. Monoterpenes 1.8-cineole and α-pinene have been recognized as the major constituents of *S. ringens* essential oil from Greece and Macedonia (Šavikin et al., 2008; Tzakou et al., 2001) and camphor and borneol in Bulgarian *S. ringens* (Georgiev et al., 2013). The oil and isolated main compounds showed significant antimicrobial activity (Šavikin et al., 2008; Tzakou et al., 2001). Among 27 Macedonian medicinal plants chosen from different plant families, *Origanum vulgare*, *Melissa officinalis*, and *Salvia ringens* showed the strongest antioxidant activity and highest amount of total phenolics, flavonoids, and phenylpropanoids (Tusevski et al., 2014). Many researchers pointed out that strong antioxidant activity of *S. ringens* extracts probably was correlated to high amount of polyphenols (Coisin et al., 2012; Nikolova, 2011; Tusevski et al., 2014). Extracts and some isolated compounds from *S. ringens* root performed significant cytotoxic activity against several human carcinoma cell lines (Janicsák et al., 2007, 2011), while literature data on antimicrobial activity of extracts were not available till now.

Taking into account the lack of comprehensive research data on of *S. ringens* herb, especially those growing wild in Macedonia, the aim of the present study was to investigate chemical composition and biological activities of its essential oil and extracts.

2. Material and methods

2.1. Standards and reagents

Methanol, ethanol, distilled water, glacial acetic acid, hydrochloric acid, hexane, dichlormethane, and ethyl acetate were purchased from Zorka Pharma, Šabac (Serbia). Gallic acid, quercetin, ascorbic acid, 2(3)-*t*-butyl-4-hydroxyanisole (BHA), 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) 2,2-dyphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), potassium acetate ($C_2H_3KO_2$), potassium-persulfate ($K_2S_2O_8$), sodium carbonate anhydrous (Na_2CO_3), alluminium nitrate nonahydrate ($Al(NO_3)_3 \times 9H_2O$), sodium acetate ($C_2H_3NaO_2$), iron(III) chloride ($FeCl_3$), iron(II)-sulfate heptahydrate ($FeSO_4 \times 7H_2O$) and Folin–Ciocalteu phenol reagent were purchased from Sigma Chemicals Co. (USA). The phenolic compounds standards were from Merck (Germany). All chemicals used in experimental procedure were of analytical grade purity.

2.2. Plant material

Aerial parts of the *Salvia ringens* Sibth. & Sm. are collected during the flowering period in July of 2012 at Krivolak locality (Macedonia). Voucher samples are stored in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade BEOU; voucher No. (16671).

2.3. Essential oil isolation

Air-dried aerial parts of *S. ringens* were grounded. Essential oil was isolated by hydrodistillation using a Clevenger type apparatus, according to the procedure I of the Yugoslavian Pharmacopoeia (1984).

2.4. Preparation of the extracts

Extracts were prepared from whole aerial plant parts using two parallel extraction procedures. Dry plant material was grounded into small pieces (2–6 mm) in the cylindrical crusher. First, portion of 10 g of plant material was successively extracted by 100 mL of dichloromethane, ethyl acetate, and methanol, according to procedure of Şenol et al. (2010) and Orhan et al. (2013). Second, portion of 10 g of plant material was individually extracted by 100 mL of solvent (ethanol and hot distilled water). In both cases, extraction was performed by classic maceration during 24 h at room temperature (10% w/v). The mixture was exposed to ultrasound 1 h before and after 24 h of maceration to improve extraction process (Veličković et al., 2007; Glišić et al., 2011). Subsequently, extracts were filtered through a filter paper (Whatman No. 1) and evaporated under reduced pressure by the rotary evaporator (Buchi rotavapor R-114). After evaporation of the solvent, the obtained crude extracts were stored in the fridge at +4 °C for further experiments.

2.5. Essential oil analysis

Qualitative and quantitative analysis was carried out using GC-FID and GC–MS. In the first instance model HP-5890 Series II gas chromatograph equipped with a split-splitless injector, HP-5 capillary column (25 m × 0.32 mm, film thickness 0.52 μm) and a flame ionization detector (FID), was employed. Hydrogen was used as carrier gas (1 mL min^{-1}). The injector was heated at 250 °C, the detector at 300 °C, while the column temperature was linearly programmed from 40 to 260 °C (4 °C/min). GC–MS analyses were carried out under almost the same analytical conditions, using HP G 1800C Series II GCD analytical system, equipped with HP-5MS column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas. The transfer line (MSD) was heated at 260 °C. The EI mass spectra (70 eV) were acquired in the scan mode in the m/z range 40–400. In each case, 1 μL of sample solution in ethanol (10 μL/mL) was injected in split mode (1:30). The identification of constituents was performed by matching their mass spectra and retention indices with those obtained from authentic samples and/or NIST/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data (Adams, 2001; Hochmuth, 2006). The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID; 250 °C).

2.6. HPLC analysis of extracts

The HPLC analyses of phenolic components were performed using the Agilent 1100 Series and UV-DAD (UV-diode array detector) according to procedure Veit et al. (1995). The column was an Agilent Eclipse XDB-C18, 5 μm, 150 × 4.6 mm, 80 Å. Injection volume was 15 μL of extracts in concentration of 10 mg/mL. Peak detection in UV region at 350 nm was used. The mobile phase was composed of solvent (A) 0.15% (w/v) phosphoric acid in water: methanol mixture (77:23, v/v, pH 2) and solvent (B) methanol as follows: isocratic 0–3.6 min 100% A; 3.6–24 min 80.5% A; 24–30 min isocratic; linear 30–60 min 51.8% A; 60–67.2 min 100% B. The flow rate of mobile phase was set to the 1 cm^3/min and temperature to 15 °C. Phenolic compounds in the samples were identified by comparing their retention times and spectra with retention time and spectrum of standards for each component. Identification of the glycoside components was based on Rf values in the HPLC chromatogram.

2.7. Determination of total phenolic content

The total phenolic content of was measured using spectrophotometric method (Singleton and Rossi, 1965). The reaction mixture