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# Chemical composition, antimicrobial, and cytotoxic properties of five Lamiaceae essential oils

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ABSTRACT

The aim of this study was to assess the biological activity of essential oils from five Lamiaceae species, *Mentha piperita*, *Mentha pulegium*, *Lavandula angustifolia*, *Satureja montana* and *Salvia lavandulifolia*, for their chemical composition, antimicrobial and cytotoxic properties. Gas chromatography coupled with FID and MS of essential oils revealed menthol (47.5%), pulegone (68.7), linalool (40.3%), thymol (44.6%), and camphor (29.1%) to be major oils' components, respectively. Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method. Seven bacterial species, representing clinical specimens, including *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, and *Enterecoccus feacalis*, and fifty eight clinical oral *Candida* spp. isolates along with three reference strains were used in experiment. All essential oils (EOs) exhibited a significant antimicrobial activity against all tested microorganisms; the oil of *S. montana* proved to be the most potent one (MIC 30.0–630.0 μg/mL, MBC 60.0–250.0 μg/mL; MIC 0.9–1.0 μg/mL; MFC 1.0–3.0 μg/mL). In addition, the oil also revealed the highest cytotoxic activity against tested cell lines, presenting $IC_{50}$ values from 40.13 to 65.51 μg/mL, with mild selectivity towards HeLa cells observed in regard to the normal cell line (MRC-5). In addition to the herbs traditional use in food and pharmacy, results of this study proved the great potential of their essential oils for application in oral disease and anticancer treatments.



## 1. Introduction

Medicinal plants present a rich source of new biologically active compounds. Diverse herbs, vegetables, fruits and spices have been used in traditional medicine and, apart from their well-known antimicrobial; many researchers have found a variety of new biological activities, including antitumoral activity (Yoo et al., 2007).

*Candida albicans* is the opportunistic pathogen that normally inhibits the gastro-intestinal tract of humans (Back-Brito et al., 2009). Under altered conditions, *Candida* spp. commensals become pathogens and cause infections ranging from mucous membrane infection, such as pseudomembranous candidiasis and denture-induced stomatitis (Lam et al., 2012), to life-threatening systemic diseases, particularly in immunocompromised patients with AIDS, cancer and *diabetes mellitus* (Seneviratne et al., 2008). In addition, it is apparent that many other oral conditions, including various forms of human periodontal diseases, may involve mixed-species infections, mainly caused by *C. albicans* and bacteria (Jenkinson and Douglas, 2002). Recent reports by researchers indicate that presence of yeasts in the oral cavity has also been linked with oral carcinogenesis (Rautemaa et al., 2007). According to the literature, more than 70% of all cancer deaths occurred in low- and middle-income countries which are also connected with poor oral hygiene (Cancer Research, 2011, Cecchini et al., 2012). This growing trend indicates that there is a crucial need for highly efficient anticancer agents with no side effects, and at an affordable cost. The vast structural diversity of natural compounds of plant origin provides unique opportunity for discovering such a new drug.

All of the tested essential oils, *Mentha piperita* (peppermint oil), *Mentha pulegium* (pennyroyal oil), *Lavandula angustifolia* (lavender oil), *Satureja montana* (winter savory oil), and *Salvia lavandulifolia* (Spanish sage oil), were analyzed in order to determine their

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chemical composition. Although, previous studies also reported chemical constituents of the oils investigated herein, we considered quite important to determine composition of our oils, due to common variations in the oils composition, affected by various factors (different origin of plant material, different industrial procedures employed in order to obtain commercial EOs, *etc.*). Further, this study aimed to investigate the biological activity of EOs from five Lamiaceae species. The oils were submitted to bioactivity evaluation by measuring *in vitro* antimicrobial potential against clinical oral isolates of bacteria and *Candida* spp. for possible application. Even though, the antimicrobial activity of selected species has already been demonstrated (Cavanagh and Wilkinson, 2002; Soković et al., 2009; Djenane et al., 2011), there are only few quantitative data (minimal inhibitory concentration or minimal bactericidal/fungicidal concentration) related to the activity of the oils against the clinical specimens. In addition, toxicity of the oils to tumor and non-tumor cells was also evaluated. The aim of this study is to make a step forward in science of using essential oils as alternatives, in microbial control and cancer therapy in humans.

## 2. Materials and methods

### 2.1. Essential oils

Five EOs from Lamiaceae family were used in this study, and all of them were purchased as commercial samples, as follows: *Mentha x piperita* L. oil from "Scents & Sensibility ltd.", USA; *Mentha pulegium* L. oil from "Athens Herbal Pharmacy", Greece; *Lavandula angustifolia* Mill.oil from "Rico Holding Ljubinje", Bosnia and Herzegovina; *Satureja montana* L. oil from "Elmard.o.o.", Bosnia and Herzegovina; *Salvia lavandulifolia* Vahl. oil from "Kirka Corporation d.o.o. Kirka Pharma", Serbia.

### 2.2. Essential oil analysis

Procedure used for GC–FID and GC–MS analyses complies with standards set for Gas Chromatography of essential oils (ISO 7609:1985; ISO 11024-1:1998; ISO 11024-2:1998).

GC–FID analysis was performed on GC Agilent Technologies 7890A apparatus, equipped with the split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (30 m × 0.32 mm, film thickness 0.25 μm) and fitted to flame-ionization detector (FID). Operating conditions were as follows: carrier gas was H2 (1 ml/min/210 °C); temperatures of injector and detector were set at 250 and 280 °C, respectively, while the column temperature was linearly programmed 40–260 °C at 4 °C/min. Solutions of essential oils' samples in ethanol (*ca.* 1%) were consecutively injected by ALS (1 μl, split-mode). The percentile presence of components in essential oils' samples was calculated from the peak areas obtained in the area-percent reports (obtained as a result of standard processing of chromatograms) without correction factors, using normalization method.

The GC/MS was performed on HP G 1800C Series II GCD analytical system equipped with HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm). Carrier gas was He (1 ml/min). Other chromatographic conditions were as those for GC–FID. Transfer line was heated at 260 °C. Mass spectra were recorded in EI mode (70 eV), in a range of $m/z$ 40–450. Solutions of essential oil samples in ethanol (*ca.* 1%) were consecutively injected by ALS (0.2 μl, split mode).

The identification of essential oils' components was based on matching of their mass spectra peaks with those from Wiley 275 and NIST/NBS libraries. The experimental values for Kovats' retention indices (RI) were determined by using calibrated Automated Mass Spectral Deconvolution and Identification System software AMDIS (ver. 2.1.), compared to those from available literature (Adams, 2009), and they were used as additional tool to support MS findings.

### 2.3. Microorganisms

Following seven clinical oral isolates, *Streptococcus pyogenes* (IBR S004), *Streptococcus mutans* (IBR S001), *Lactobacillus acidophilus* (IBR L001), *Streptococcus salivarius* (IBR S006), *Streptococcus sangunis* (IBR S002), *Streptococcus sanguis* (IBR S005), *Enterecoccus feacalis* (IBR E001), *Pseudomonas aeruginosa* (IBR P001), and one reference strain, *Staphylococcus aureus* (ATCC 25923), were used in the study. In antifungal assay, fifty-eight clinical isolates of *Candida* spp., as well as two references strains (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750) were used. The reference strains were obtained from the Collection of the Laboratory of Mycology at the Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The bacteria species were maintained in Mueller Hinton Agar and Tryptic Soy Agar (MHA, TSA, Merck, Germany). Strains of *Candida* spp. were maintained on Sabourand Dextrose Agar (SDA, Merck, Germany). All clinical oral isolates were obtained by rubbing a sterile cotton swab over oral mucosa from patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia.

The colonies obtained were analyzed for morphological, cultural and physiological characteristics. Proper identification of oral bacteria (Cecchini et al., 2012) and fungi (Nikolić et al., 2012) colonies were performed.

### 2.4. Antimicrobial activity

Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method in 96 well microtitre plates described by Douk et al. (1995) and EUCAST (2002) with some modifications. Briefly, fresh overnight cultures of bacteria were adjusted with sterile saline to a concentration of $1.0 \times 10^5$ CFU/well for bacteria and fungi, respectively. EOs were added in TSB medium for bacteria, SDB medium for *C. albicans*. The microplates were incubated for 24 h at 37 °C for bacteria and 48 h at 37 °C for yeasts. The MIC was defined as the lowest concentration of EO inhibiting the visible growth of the test strain. The MIC/MBC values for bacteria and yeasts were detected following the addition of 40 μL of p-iodonitrotetrazoliumviolet (INT) 0.2 mg/mL (Sigma I8377) and incubation at 37 °C for 30 min (Tsukatani et al., 2012). The MBCs/MFCs were determined by serial subcultivation of 10 μL into microtiter plates containing 100 μL of broth per well and further incubation for 24 h at 37 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. Positive controls, antibiotics (Hexoral®, Streptomycin) and antimycotic (Fluconazole), were used in both experiments.

### 2.5. Cell culture

Human cervix carcinoma cells (HeLa), lung adenocarcinoma cells (A549), and human fetal lung fibroblast cells (MRC-5) were maintained as monolayer culture in the Roswell Park Memorial Institute (RPMI) 1640 nutrient medium (Sigma Chemicals Co., USA). RPMI 1640 nutrient medium was prepared in sterile ionized water, supplemented with penicillin (192 U/mL), streptomycin (200 mg/mL), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) (25 mM), L-glutamine (3 mM) and 10% of heat-inactivated fetal calf serum (FCS) (pH 7.2).