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Chemical composition and biological activity of *Gaultheria procumbens* L. essential oil

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ABSTRACT

The aim of this study was to examine chemical composition and biological activity of *Gaultheria procumbens* L. essential oil (EO) against food spoilage and oral microorganisms. The components of EO were identified by GC–MS. Antimicrobial activity was determined against food spoilage (five bacteria and six fungal species) and oral microorganisms (eight bacteria and thirty two fungal species) by microdilution and microplate biofilm assay, antioxidant activity was tested using the persistent free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH), while antiradical activity was examined by fluorescence spectroscopy and electron paramagnetic resonance spectroscopy (EPR). GC–MS.analysis showed that methyl salicylate (96.90%) was the main component of the oil. Essential oil inhibited the growth of all microorganisms tested, i.e. food and oral bacteria and fungi, respectively (MIC 0.18–3.00 mg/ml and MBC 1.25–4.00 mg/ml; MIC 0.73–5.00 mg/ml and MFC 2.92–26.67 mg/ml); The oil effectively inhibited the biofilm formation of oral *Streptococcus mutans* and *Candida albicans* as well (MIC 25.00 MBC 50.00 mg/ml; MIC 12.50, MFC 50.00 mg/ml). In addition, oil exhibited a dose-dependent DPPH-radical-scavenging activity with IC_{50} value of 30.61 mg/ml. The specific fluorescence probe *2-[6-(4′-amino)phenoxy-3H-xanten-3-on-9-yl] benzoic acid* (APF) and the the spin trap *5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide* (DEPMPO), capable for simultaneous detection of different free radical specie were used in antiradical activity of the oil measurements. Oil showed a moderate antiradical activity, reducing quantity of produced hydroxyl radicals to about 20% of initial value. This study succeeds in creating directly comparable and quantitative data for the oil unsufficiently examined so far.

1. Introduction

Traditional plant medicine currently holds an important place in the treatment of patients, primarily in developing countries, whereas the use of essential oils is of particular and growing interest (Kalemba and Kunicka, 2003). At the same time scientists have recognized the significance of ethnomedicine as an effective tool for the treatment of a broad range of severe human diseases (Bakkali et al., 2008), finding various biologically active compounds in these natural remedies such as mono- and sesquiterpenes, carbohydrates, alcohols, ethers, aldehydes and ketones (Newmann et al., 2000).

Increase resistance to antibiotics and the fact that many of them can be toxic and cause different side effects makes plant-based alternatives of great subject of interest. A serious number of essential oils showed antimicrobial activity against pathogenic microbes, some better than others. Further investigations have indicated that overexposure to antibiotics in general may encourage the carriage of resistant strains among the oral and gastrointestinal flora, especially in children. In addition, pharmacokinetic characteristics and pharmacodynamic properties dictate not only antimicrobial response and clinical outcome, but might have an increasing impact on the emergence of resistance (Kastner and Guggenbichler, 2001).

Recently, interest in the study of oral diseases such as dental caries and candidiasis being the most common one, has markedly increased mainly because of its importance and association with human immunodeficiency virus (HIV) infection, but also due to its relation with potentially malignant lesions of oral mucosal. By some reports the economic impact of oral diseases is considerable with up to 10% of public health expenditure (Petersen et al., 2005). Many authors have studied the characteristics of oral mucosa in immunocompromised patients in order to find the differences in

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immunologic reactions to the development of candidiasis. There are a number of oral lesions that are clearly associated, more often than others, with either candidial infestation or frank invasion. The yeast-like fungus *Candida albicans* and a few other Candida species are capable of producing skin, mucous membrane, and internal infections. The organism lives with the normal flora of the mouth, vaginal tract, and gut, so the results of culture analysis must be interpreted carefully. Pregnancy, oral contraception, antibiotic therapy, diabetes, skin maceration, topical steroid therapy, certain endocrinopathies, and factors related to depression of cell-mediated immunity may allow the yeast to become pathogenic and produce budding spores and elongated cells (pseudohyphae) or true hyphae with septate walls. (Vuckovic et al., 2004)

As the number of reported cases of food-associated infections continues to increase, food safety is a fundamental concern of both consumers and the food industry. As aromatic plants, herbs and spices have been used for ages both as flavoring agents and as preservatives of food, they may be effective sources of biodegradable fungitoxicants without harmful side effects. In the present study, a search was made to find essential oil that could safely be used as natural alternatives for chemical agents (Sokovic et al., 2009).

In search for natural products that may be suitable and considering the multiple therapeutic properties of plant essential oils and their extensive usage against infectious diseases both in traditional and modern medicines, this investigation has been made with a primary objective to determine the biological potential of *Gaultheria procumbens* essential oil.

G. procumbens (wintergreen) is an evergreen shrub from Ericaceae family native to eastern parts of North America. The oil of this plant species is rich in methyl salicylate and has a pleasant wintergreen flavor. Its oil is used for flavoring chewing gums (Teaberry gum), candies, toothpaste, and mouthwashes (Facciola, 1998). It is also used in the treatment of cellulites, a bacterial infection that causes the skin to become inflamed (Genders, 1977).

Reactive oxygen species (ROS) are generated by normal metabolic processes and could be formed through different mechanisms. Damages induced by ROS include: DNA mutation, protein oxidation and lipid peroxidation, contributing to the development of cancer, diabetes, atherosclerosis, inflammation, and premature aging (Aas et al., 2005). About 95% of the pathologies observed in people above 35 years are associated with production and accumulation of free radicals. However, antioxidants of essential oils could allow variety of prospective solutions for the aforementioned medicinal conditions (Slots et al., 1990).

The present work deals with identification of EO components by gas chromatography–mass spectrometry analysis (GC–MS., due to better understanding of its bioactivity. The antimicrobial effect was carried out to estimate the influence of the plant on certain microbes such as food spoilage and oral microorganisms according to its traditional medicine properties. Antioxidant and antiradical activity assessment were also carried out in *in vitro* conditions. The antioxidant activity of *G. procumbens essential* oil was determined using DPPH (*2,2-diphenyl-1-picryl hydrazyl*) radical, while anti hydroxyl radical activity (•OH) was examined by fluorescence probe APF (*2-[6-(4′-amino)phenoxy-3H-xanten-3-on-9-yl] benzoic acid*), the derivate of fluorescein. In order to avoid possible errors caused by autofluorescence, quenching or changes in quantum yield, antiradical activity was examined by electron paramagnetic resonance spectroscopy (EPR) and specific spin-trap DEPMPO. The advantage of a spin trap DEPMPO lies in its structure which allows obtaining characteristic EPR signals for different free radical species. It was important to emphasize the antiradical activity to •OH radical which is is present in any living system in contrast to •DPPH, •$ABTS^+$ or •DMPD commonly used in most standard test but absent in biological systems.

Nevertheless, as far as we know, this is the first report on chemical composition antimicrobial and antioxidant activity of essential oil from *G. procumbens*.

2. Materials and methods

2.1. Essential oil

G. procumbens L. (EO) was purchased from the Institute for Medicinal Plants "Dr. Josif Pančić", Belgrade, Serbia. Two commercial mouthrinses were purchased: Tebodont® (Dr. Wild & Co. AG, Switzerland): aqua, glycerin, propylene glycol, PEG-40-hydrocarbonated castor oil, *Melealeuca alternifolia*, sodium saccharin, dipotassium phosphate, aroma, limonene, linalool; Hexoral® (Hemofarm A.D., Serbia): ethanol 96%, polysorbate 80, citric acid, no water, levomenthol, methyl salicylate, saccharine-sodium, essential oil of *Mentha* sp., essential oil *of Anis* sp., essential oil of *Chamommila* sp., essential oil of *Syzygium clove*, essential oil of essential oil of *Eucaliptus* sp., amaranth (E 123), ultra-pure water.

2.2. Essential oil analysis

The EO sample was diluted in ethanol (1 μl) and injected in a split-mode (1:30). Gas chromatography was performed on GC Agilent Technologies 7890A apparatus, equipped with the split–splitless injector attached to HP-5 column (30 m × 0.32 mm, film thickness 0.25 μm) and fitted to flame-ionisation detector (FID). Operating conditions were as follows: carrier gas was H_2 (1 ml/min/210 °C); temperatures were set as follows: injector at 250 °C and detector at 280 °C, while the column temperature was linearly programmed 40–260 °C at 4 °C/min. The percentage composition was computed from the peak areas, without correction factors.

The GC–MS.was performed on HP G 1800C Series II GCD analytical system equipped with HP-5MS 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.

The identification of individual constituents was accomplished by comparison of their spectra with those from available MS libraries (NIST/Wiley) and by comparison of their experimentally determined retention indices (calibrated AMDIS), with data from the literature (Adams, 2009).

2.3. Microorganisms

The tested microorganisms included the following food spoilage group; Gram negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterococcus cloacae* (human isolate) and the following Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973) and *Staphylococcus aureus* (ATCC 6538). Six clinical oral isolates were also included, specifically, *Streptococcus mutans* (IBR S001), *Streptococcus sanguis* (two strains, IBR S002 & IBR S003), *Streptococcus pyogenes* (two strains, IBR S004 & IBR S005), *S. aureus* (ATCC 25923), *P. aeruginosa* (IBR P001) and *Lactobacilus sp.* (IBR L002). In antifungal assay, six fungi from food spoilage ad food contaminators and mycotoxin producers were used: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (ATCC 204305), *Aspergillus niger* (ATCC 1015), *Aspergillus flavus* (ATCC 9643), *Penicillium funiculosum* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112) as well as thirty one clinical oral isolates of *C. albicans* and reference strain of *C. albicans* (ATCC 10231 strain). The reference strains were obtained from the Laboratory of Mycology at the Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia. The