Antimicrobial activity of *Thymus longicaulis* C. Presl essential oil against respiratory pathogens

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1109

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1. Introduction

Essential oils produced by aromatic plants have been used from ancient times as antiseptics and anti-infectious agents. Since the discovery and development of chemotherapeutic agents, especially antibiotics, the use of essential oils has been reduced, but such oils have been used traditionally for the prevention and therapy of respiratory tract infections [1]. Respiratory infections are the most frequent reason for primary health care consultation. Epidemiological studies indicated that one fourth to one third of patients visiting general practitioners suffer from acute respiratory infections. Paediatric respiratory tract infections are especially associated with significant morbidity and mortality. Additionally, antimicrobial resistance among respiratory tract pathogens has become an increasing problem world-wide [2,3]. Since infections with respiratory tract pathogens are common in all age groups, their contribution to the total consumption of antimicrobial agents in a population is therefore significant. Despite the many infectious disease control and surveillance...
programs, the medical community has not been able to stop the global spread of resistant microorganisms. The increasing antimicrobial resistance in major respiratory pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus* and Gram-negative bacteria has severely restricted the treatment options. Respiratory infections caused by multidrug-resistant bacteria are associated with a greater likelihood of inappropriate antimicrobial therapy and poor clinical outcome [4,5]. The quest for a solution to the global problem of antibiotic resistance has often focused on finding new antimicrobial compounds from a variety of natural sources. In this regard, plant essential oils may offer a great potential for the development of novel antibacterial therapies and complementary treatments.

The genus *Thymus* (Lamiaceae) is noteworthy for the numerous wild-growing plants biosynthesizing a remarkable amount of volatile compounds. *Thymus* species are perennial, aromatic herbs and subshrubs native to Europe and North Africa [6]. They are commonly used as culinary herbs, flavouring agents and medicinal plants. Recent studies have shown strong antibacterial, antifungal, antiviral, antiparasitic, spasmyloytic and antioxidant activities of *Thymus* species. It has been considered that a part of these effects is due to the volatile constituents [7-13]. Therefore, there is a considerable research interest in the chemical composition analysis of *Thymus* essential oils and their biological activities.

*Thymus longicaulis* C. Presl is a small aromatic perennial herb and a typical representative of the Illyric-Mediterranean flora. A great number of subspecies, varieties and forms were described within this polymorphic species [14]. It is a traditional remedy for cold, flu, cough, nephritis and abdominal pain [15-18]. To the best of our knowledge, there are several reports on the essential oil analysis of different taxa of *T. longicaulis*, and the results revealed their marked chemical variations depending on genetically determined properties, localities and environmental factors [19-26]. Three reports have been published concerning the antimicrobial activity of *T. longicaulis* recently. Essential oils isolated from *T. longicaulis* of Italian origin as well as from its two subspecies *chaubardi* and *longicaulis* growing in Turkey showed activities against several strains of common pathogenic bacteria and *Candida albicans* [27,28]. Susceptibility of foodborne bacteria on *T. longicaulis* essential oil was also assessed [29].

To find new antimicrobial resources, the present study aims to evaluate the composition of the essential oil of *T. longicaulis* growing in Croatia and its *in vitro* antimicrobial activity against major respiratory tract pathogens that show increasing resistance to commonly prescribed antimicrobials. Although this group of microorganisms has shown a high susceptibility to several other essential oils [1,30], activity of *T. longicaulis* essential oil against clinically relevant respiratory tract pathogens has not been investigated thus far.

### 2. Experimental Procedures

#### 2.1 Plant material and essential oil isolation

Aerial parts of wild-growing *Thymus longicaulis* C. Presl were collected at the flowering stage from Jasenice (Central Dalmatia, Croatia, 130 m a.s.l.) in May 2010. The plant sample was authenticated by Department of Botany and Botanical Garden, Faculty of Science, University of Zagreb, Croatia, where a voucher specimen of the plant is deposited (No. 819-4). The aerial parts of the plant were dried at room temperature, then crushed and hydrodistilled for 3 hours using Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored at 4°C [31].

#### 2.2 Gas Chromatography/Mass Spectrometry analysis (GC/MS)

The essential oil was analysed by GC/MS on CP-3800 gas chromatograph coupled with Saturn 2000 mass selective detector (Varian, Palo Alto, USA), equipped with CP Sil-8 capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Operating conditions were as follows: carrier gas, helium at a flow rate of 1 mL/min; column temperature, 60-250°C at a rate of 4°C/min; injector temperature, 280°C; injected volume 0.1 μL; split ratio, 1:100. Mass spectra were recorded at 70 eV and were scanned in the range 45-350 uma. The individual components of the essential oil were identified by comparison of their retention indices with either those of the literature [32] or with those of authentic compounds available in our laboratory. The retention indices were determined in relation to a homologous series of *n*-alkanes (*C*<sub>8</sub>-*C*<sub>20</sub>) under the same operating conditions. Further identification was made by comparison of their mass spectra with either those stored in NIST2000 and in-house library or with mass spectra from the literature [33,34]. Relative amounts of the essential oil components were obtained by peak area normalization.

#### 2.3 Antimicrobial assays

Activity of *T. longicaulis* essential oil against respiratory pathogens was evaluated by disc diffusion method and microdilution broth susceptibility assay. Fresh clinical bacterial (*Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*) and yeast