ABSTRACT

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Title of Doctoral Thesis **The using of GC and SPME in the analysis of natural products**

Due to the wide of use of SPME and GC in the analysis of natural products, this research is focused on the field of botany and ecology on the study of volatile compounds from plants.

The material for a study were two species of Lamiaceae family, Melittis melissophyllum L. subsp. melissophyllum a Teucrium flavum L. subsp. flavum. In the theoretical part are described as botanical as farmacognostical characteristics of the family Lamiaceae and the species under the study, respectively. Furthermore, the discussion on volatile substances of natural, plant origin including chemical characteristics, biological activity and methods of analysis sensu lato is given.

The essential oil of flowering aerial parts of Melittis melissophyllum subsp. melissophyllum (Lamiaceae), growing in central Italy, was obtained by steam distillation and characterised by GC–FID and GC–MS. The total of 48 components were identified (94.7 -95.0%). The main component was the mushroom-like flavour 1-octen-3-ol (43.6 - 54.2%). The result suggests,that the plant could be considered as a new natural source of this molecule used for example in the food industry. The subsequent performing headspace analysis (HS-GC), suggested that this aromatic compound is present in the plant only at low concentration and its amount increases by the steam distillation of this material (SD). Increasing importance in the analysis of volatile compounds from plants, with respect to the sampling, has assumed the headspace (HS) - Solid Phase Microextraction (SPME). The goal of research was to choose the most effective SPME fiber to optimize the conditions of sampling and comparison of HS-SPME with other extraction techniques, steam distillation (SD). On this basis, the analysis of volatile compounds Teucrium flavum L. subsp. flavum, growing in central Italy, was carried out by means both by steam distillation (SD) and Headspace – Solid Phase Microextraction (HS-SPME) coupled with GC / FID and GC / MS. A total of 102 substances were identified from the essential oil obtained by steam distillation (99.0 - 99.3% of the all ingredients of the analyzed oil). The sesquiterpene hydrocarbons constituted the major fraction (48.5 – 49.4%), with the apple-like flavour (Z,E)-α-farnesene being the major component. During HS-SPME analysis three kinds of fibers was tested: 100 μm polydimethylsiloxan (PDMS), 65 μm PDMS / divinylbenzen (DVB) and 50/30 μm DVB-Carboxen-PDMS. The best fibre was found to be PDMS when working in the following conditions: 60°C temperature, 30 min extraction time, 30 mg sample amount, 1mm sample particle size. By HS-SPME method was identified 76 (dry sample) resp. 66 (fresh sample) of various volatiles (95.8 – 97.8%). In addition, it was found that the presence of water in the sample can enhance the absolute quantity of alcoholic compounds such as 1-octen-3-ol, and reduce the presence of esters, such as methyl geranate. Using this method, the HS-SPME samples as of the whole flowering aerial part of plants as of the different their parts and the components of flowers were analyzed. The flower calyx shows to be the largest contributor – in terms of volatile substances – to plant aroma. SPME technique thus allows to analyze only targeted parts of plants, too. Therefore, it may be appropriate for sampling as in the industrial applications of aromatic plants or on the field of chemical ecology.

Plants emit volatile organic compounds (VOCs) that play important roles in their interaction with the environment and have a major impact on atmospheric chemistry. The development of static and dynamic techniques for headspace collection of volatiles in combination with gas chromatography–mass spektrometry analysis has significantly improved our understanding of the biosynthesis and ecology of plant VOCs.