

GRASSE • SEPTEMBER 10TH - 13TH, 2006

37th INTERNATIONAL SYMPOSIUM
ON ESSENTIAL OILS

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2006



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Comparative analysis of the volatile constituents of *in vitro* and *ex vitro* plants of *Petiveria alliacea* L.

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Petiveria alliacea L. (Phytolaccaceae) is an herbaceous perennial herb that reaches up to 1.5m height and is characterized by erect branches, alternating leaves and very small white flowers. It occurs in tropical and sub-tropical regions, and is commonly found in Brazil in the Amazon region. Although the species is traditionally used in popular medicine due to several pharmacological properties, the continuous use of the root powder brings about neurological effects such as super-excitation, insomnia and hallucinations, followed by convulsions, paralysis and death. Previous phytochemical investigations have reported the presence of sulphur containing substances on the flowers of this plant, which are responsible for the characteristic garlic aroma of the species. In the present work tissue culture protocol for *P. alliacea* was established for comparison of the phytochemical profile between *in vitro* and *ex vitro* plants. *In vitro* propagation was achieved through the culture of nodal segments on MS medium. Rooting of shoots induced in half strength MS supplemented with IAA 0,6 µM. Rooted shoots were transferred to green house and morphological abnormalities were not observed in the plants. The volatile oils were obtained by Simultaneous Distillation and Extraction (SDE) from *ex vitro* comminuted fresh leaves and roots and from fresh leaves and roots of the *in vitro* cultivated plantlets, for four hours. The solvent used was dichloromethane and for GC quantification 0,32µl of tridecane (0, 24µg) were added as internal standard. Analyses were performed in a HP 5890 gas chromatograph equipped with a FID detector and a HP5 fused silica capillary column (30m X 0.25mm X 0.25µm), using H₂ (1.0mL/min) as carrier gas. The injector temperature was kept at 250°C and the oven temperature was programmed from 60 to 240°C at 3°C/min. Pure oils (0.03µL) were injected in split mode (100:1). The GC-MS analyses were recorded using an Agilent 5973N GC-MS system, using the same column and chromatographic conditions, but helium as the carrier gas. The constituents of the oils were identified by comparing their mass spectra with those in a spectral database (Wiley 6th ed) and by their retention indices (RI). A standard solution of n-alkanes (C₇-C₂₆) was used to obtain the retention indices.

Forty two different compounds were identified in the essential oils of *in vitro* and *ex vitro* structures of *P. alliacea*, many of them were present only in trace amounts (below 0.1%). The comparative analysis of the essential oils from both leaves and roots showed differences between them. However, benzaldehyde and a series of unsaturated long chain fatty acid methyl esters were present in all analyzed samples. Also, sulphur containing substances such as bis-phenylmethyl)-disulphide, isothiazol (1,2-thiazol), 2-thiopropene, dimethyl sulphide, ethylene disulphide and 2,3-dimethylthiirane were identified in both leaves and roots of *in vitro* and *ex vitro* plants. This is in accordance with the fact that not only the flowers of this plant smell like garlic, but also all the plant parts do. Sesquiterpenes were found mainly as trace components of *ex vitro* roots essential oil.