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COMPARATIVE ANALYSIS OF THE OIL AND SUPERCRITICAL CO₂ EXTRACT OF *ACORUS CALAMUS*

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Nowadays the interest in phytoproducts has grown significantly in Estonia and worldwide. Each third product on the international pharmaceutical market has a phyto origin. Nevertheless, most of essential oil plants are not yet well studied.

The aromatic rhizomes of *Acorus calamus* are used extensively in traditional medicine worldwide. *A. calamus* includes four cytotypes: diploid (North America, no β -asarone), triploid (Europe, 5-20% β -asarone), tetraploid (East Asia, India and Japan, β -asarone up to 70%) and hexaploid (Kashmir Region, β -asarone content of 5 %.). The chemical composition of essential oils depends on plant ploidy. The compound, β -asarone, is of moderate acute toxicity. Because of this property its use in digestive medicine was discontinued in many countries. β -Asarone has been demonstrated to induce duodenal and liver cancer in rats, central nervous system inhibitory, sedative and hypothermic, unscheduled DNA synthesis in hepatocytes and immunosuppressive effects.

The simultaneous distillation and extraction micro-method (SDE) and supercritical fluid extraction (SFE) were used to isolate the essential oil from the plant's samples. The capillary gas chromatographic (GC/FID) analysis was applied to the identification of oil components and determination of their content in the oil. Supercritical extraction was performed on a self-completed equipment under pressure of 100 bar and temperature 50°C. GC analysis was carried out using a chromatograph with FID on two fused silica capillary columns with bonded stationary phases SPB-5 (poly(5%-diphenyl-95%-dimethyl)siloxane) and SW-10 (polyethylene glycol). The identification of oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data and literature data. The results obtained were confirmed by GC/MS.

The yield of calamus oil was in the range of 0.9-3.3% for SFE and 1.4-3.3% in the case of SDE. In the studied oils, 72 compounds were identified, representing more than 95% of the total oils. The extracts obtained by SFE contained mainly β -asarone, isoshyobunone, shyobunone, preisocalamendiol, spirodecanone, acorenone, acarone. A comparison with the SDE oil did not reveal any big differences, except for acarone (13.3% versus 2.8%), acorenone (12.5% versus 22.4%), preisocalamendiol (15.7% versus 8.1%) and spirodecanone (5.1% versus 0.9%). β -Asarone (SDE 85.3%, SFE 82.8%) was the major constituents in one sample (characteristic for tetraploid *A. calamus*). Two samples (triploid type) had lower percentage of β -asarone (SDE 9.3-10.2%, SFE 5.4-7.6%).

Although a lot of works on analysis of calamus oil have been reported, the detailed GC analysis of Estonian calamus has not been carried out. In the present study the composition of the essential oil and supercritical CO₂ extract of *A. calamus* rhizomes growing in Estonia were investigated. This work is the first study using SFE from calamus rhizomes of β -asarone rich samples.