

# Localisation and detection of cannabinoids in isolated *C. sativa* L. trichomes

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*Cannabis sativa* L. is an annual, dioecious herb, belonging to the family of Cannabaceae and originating from Eastern and Central Asia. Phytocannabinoids (cannabinoids), a unique group of terpenophenolics possessing alkyl-resorcinol and monoterpene moieties in their molecular structure are considered the most responsible compounds for the biological activities of *Cannabis sativa* L. (Figure 1) More than 100 cannabinoids have been identified and structurally elucidated, including recently isolated new entities. Because of their psychoactivity,  $\Delta^9$ -tetrahydrocannabinol (THC) and its related acid (Figure 2) and cannabidiol (CBD) are the most studied and interesting compounds of the class. Here we analysed by Laser Dissection Microscopy, LC-MS and LC-NMR pattern and localization of THC production in trichomes.

Trichomes, especially the capitate-stalked glandular hairs, are well known as the main sites of cannabinoid and essential oil production of *C. sativa*. In this study the distribution and density of various types of *Cannabis sativa* L. trichomes (Figure 1, right), have been investigated by scanning electron microscopy.



Figure 1. *Cannabis sativa* L. (left) and capitate stalked trichome (right)

Furthermore, glandular trichomes were isolated over the flowering period (8 weeks) by laser microdissection (LMD) (Figure 3) and the cannabinoid profile analyzed by LCMS. Cannabinoids were detected in extracts of less than 200 cells collected from capitate-sessile and capitate stalked trichomes and separately in the gland (head) and the stem of the latter.  $\Delta^9$ -Tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabigerolic acid

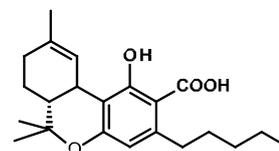


Figure 2.  $\Delta^9$ -tetrahydrocannabinolic acid (THCA)

(CBGA) were identified as most-abundant compounds in all analyzed samples while their decarboxylated derivatives,  $\Delta^9$ -tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG), co-detected in all samples, were present at significantly lower levels. Cannabichromene (CBC) along with cannabiol (CBN) were identified as minor compounds only in the samples of intact capitate-stalked trichomes and their heads harvested from 8-week old plants. Cryogenic nuclear magnetic resonance spectroscopy (NMR) was used to confirm the occurrence of major cannabinoids, THCA and CBDA, in capitate-stalked and capitate-sessile trichomes. Cryogenic NMR enabled the additional identification of cannabichromenic acid (CBCA) in the dissected trichomes, which was not possible by LCMS as standard was not available. The hereby documented detection of metabolites in the stems of capitate-stalked trichomes indicates a complex biosynthesis and localization over the trichome cells forming the glandular secretion unit.

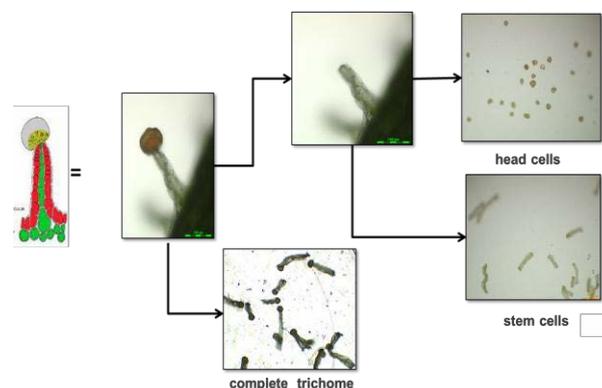


Figure 3. Schematic view on the laser dissection of trichomes from *C. sativa* L.

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## Publications:

- ✓ Happyana, N., Agnolet, S., Schneider, B., Kayser, O. (2012) Analysis of cannabinoids in laser microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. *Phytochemistry* 87:51-59
- ✓ Happyana, N., Muntendam, R., Kayser, O. (2012) Metabolomics as bioanalytical tool for characterization of medicinal plants and their phytomedicinal preparations, in *Pharmaceutical Biotechnology 2<sup>nd</sup> ed.* (eds. Kayser, O., Warzecha, H.) pp. 527-552