Summary

The modern analysis of natural products by highthroughput-screening (HTS) could be more faster and efficient by specific selection of the screeningobjects.
A combination of the HTS with a preconnected HPLC-system can increase the effectiveness of this method because of the possibility to directly assign a bioactivity in HTS to a specific HPLC peak. The advantage of this combination is that isolation of a compound only starts when it has shown activity in HTS previously.
The systematic search for new actives in medicine with this revolutionary screening technology has induced a BMBF-Project (Title: „Validierte Lead/Target-Systeme – eine horizontale integrierte Verbundstruktur zur automatisierten Pharma-Wirkstofffindung“) in which these work was integrated. The aim was not only to supply as many natural extracts with promising active candidates as possible but also to limit the number of extracts which will be used in the lavish and costly HTS (or HPLC/HTS).
Therefore a prescreening system was established allowing the determination of several bioassays directly on the thin-layer plate as e.g.:

- Inhibition of Cholinesterase
- DPPH-activity
- antibacterial activity with Bacillus subtilis, E. coli and S. aureus
- Haemolytical effects with blood-agar

With the development and improvement of such easy, quick and economical test systems we create a platform to screen a lot of plants and fungi of their pharmacological activity in a short time without expensive technical instruments.
The evaluation of these data of 50 organisms used in these bioassays led to a selective input to the HTS of 23 extracts. 16 total extracts (not preseperated by HPLC) thereof show activity in a caspase-assay. Three further extracts out of the 50 pretested were screened with the combined technique HPLC/HTS because of their strong positive effects in our established “in-situ”-assays on the TLC plates. They also showed significant effects with HIR-kinase-assay.
In addition to these 50 prescreened organisms we tested different total extracts from more than 100 organisms in HTS against the caspase-assay at EVOTEC OAI. However, most of them failed these test due to various technical problems. Those negative results let us build up our “in-situ”bioassays and in this way we could successfully increase the rate of positive hits by a useful preselection.
Apart from a specific selection for the HTS these bioassays itself offer the possibility to detect active compounds which could be isolated and subjected to structural elucidation in order to find new active lead compounds.
For example in this way we could detect and isolate a cholin-inhibiting compound (Fomajorin S) of the root-rotting fungi *Heterobasidion annosum.*

These “in-situ”bioassays have also the advantage that they can detect bioactivity of volatile compounds gained by hydro distillation, which are not detectable by HTS due to evaporating processes. In the volatile fraction of our fungi we could detect inhibition of the cholinesterase activity and radical capture activity in the DPPH-Test and measure the MS spectra of these eight active compounds.
The meaning of such a pre-screening with TLC-Bioassays was also shown by the positive effect the extract of *Heterobasidion annosum* exhibited in the HTS (HIR-kinase-assay), which allowed to assign the inhibition activity to a specific peak in the HPLC-(ESI)-MS-chromatogram.