Summary

The global prevalence of infestations with soil-transmitted helminths is estimated to be more than one billion, most of which occur in developing areas of sub-Saharan Africa, South America and South East Asia. As the number of anthelmintic drugs is very limited and resistances are starting to emerge there is an urgent need for novel drugs or alternative treatments. As many patients in endemic countries rely on traditional plant based medicine the following study aimed at the identification of medicinal plants with documented anthelmintic activity by an ethnopharmacological approach. Selected plants from this survey had to be characterized concerning phytochemical aspects including the respective active ingredients, functional aspects against relevant soil-transmitted helminths and the elucidation of the underlying mode of action on a molecular level.

An ethnopharmacological survey among traditional healers in central Ghana revealed several medicinal plants as interesting candidates for an *in vitro* screening. Ethanol-water (1:1) extracts from the leaves of *Combretum mucronatum* and the roots of *Paullinia pinnata* showed promising activities against the free-living nematode *Caenorhabditis elegans* (LC_{50} 1.9 and 2.5 mg/ml, resp.) as well as against feline roundworms (*Toxocara cati*; LC_{50} 46 and 189 µg/mL, resp.) and canine whipworms (*Trichuris vulpis*; LC_{50} 30 and 17 µg/mL, resp.) representing the animal counterparts of the most prevalent parasitic species in humans. A bioassay-guided fractionation of the leaf extract from *C. mucronatum* revealed oligomeric procyanidins as the active principle. The respective procyanidins (compounds **2, 3, 4, 6, 7, 8, 9** and **10** and oligomeric clusters of a distinct degree of polymerization from 3 to 10) were mainly composed of epicatechin building blocks connected *via* typical 4β→8 or 4β→6 interflavan linkages. Additionally, a dimeric flavan-3-ol (**5**) with an unusual interflavan linkage between position 6' of the B-ring (upper unit) and position 8 of the D-ring (lower unit) was isolated from *C. mucronatum*. A degree of polymerization of 3 was found to be the threshold for anthelmintic effects of the oligomeric procyanidins. However further increase in the molecular size of the oligomers above a degree of polymerization of 4 did not improve the activity. This finding correlates well with previously described interactions of condensed tannins with proteins. Flavonoids and fatty acids that were also present in the test extracts did not exhibit any direct anthelmintic activity but enhanced the effect of the oligomeric procyanidins.

Extracts from *P. pinnata* (EtOH-H_2O 1:1; acetone-H_2O 7:3; hot acetone) were strongly enriched in highly polymerized procyanidins, containing predominantly A-type interflavan linkages which have previously been rarely investigated for an anthelmintic activity. Fractionation of the acetone-H_2O 7:3 extract on Sephadex®-LH20 further revealed small amounts of two yet unidentified lipophilic substances that seem to enhance the nematicidal effect of the A-type procyanidins.

A protocol for the synthesis of dansylated higher oligomeric procyanidins was developed maintaining the protein binding capacity of the OPCs, which potentially allows localization of procyanidin binding sites within functional investigations.

A transcriptome analysis of *C. elegans* treated with oligomeric procyanidins from *C. mucronatum*, followed by quantitative PCR analysis for cross validation revealed insights into the molecular mode of anthelmintic action of this group of tannins. The expression of the nematode specific proteins PUD-1.1, PUD-1.2 and PUD-3 was strongly down-regulated in response to the procyanidins, which was also the case for enzymes associated with the lysosome, lipid metabolism and certain immune responses. These findings also partly overlapped with biochemical processes identified during mild stresses that have been associated with longevity, such as dietary restriction or low dose treatment with polyphenols. Up-regulated genes encoded for proteins with a variety of different functions, but a

predominant localization in cell membranes. In particular, the expression of a proline-rich orthologue (T22D1.2) of a human salivary protein which is suspected to act as a hitherto undescribed invertebrate defense against tannins, was increased up to about 900-fold by the procyanidins. This extraordinarily increased expression of T22D1.2 was observed especially in the intestine of the nematode, as shown using a gfp transcriptional fusion of the respective gene. Further, several homologues of structural proline- or histidine-rich proteins in humans were increasingly expressed as well as certain genes involved in pathogen defense and one *Caenorhabditis* specific metal responsive (*numr-1*) indicating metal induced stress. This pointed towards an interference of polyphenols with the worms' ion balance in addition to the interaction with proteins, possibly induced by chelating of polyvalent ions via the catechol function of the flavan-3-ols or binding to membrane-bound ion channels.

Overall, the findings of this study support the traditional use of *C. mucronatum* and *P. pinnata* and contribute to a better understanding of the underlying structural features affecting the anthelmintic activity of oligomeric procyanidins. Further, insights revealed into the molecular mode of action of procyanidins provide a base for the exploration of manifold potential targets for novel anthelmintics.