Summary

Introduction

Research on herbal preparations for medicinal use for the long-term therapy of chronic diseases such as chronic inflammatory bowel disease (IBD) is an essential point for modern rational phytotherapy. The use of products containing ripe fruits from *Vaccinium myrtillus* (bilberries) or bilberry extracts is getting more and more into the focus of IBD therapy. The present study thus focused on:

I) detailed phytochemical analysis of bilberries (wild blueberries) and bilberry-rich products concerning identity, and content of anthocyanins (AC) and tannins for analytical and quality specifications;

II) *in vitro* studies of AC from bilberries in cell culture models with human intestinal epithelial cell lines.

Methods

Phytochemical analysis of bilberries and bilberry-containing products were conducted using validated pharmacopoeial methods such as thin layer chromatography (TLC), HPLC, determination of tannin content, and testing for uniformity of mass. Preclinical *in vitro* investigations were performed using the human intestinal epithelial cell lines Caco-2 and HT-29 and included analysis of the effects of bilberry-AC on viability (MTT-assay), proliferation (BrdU-assay), mucin production (alcianblue-assay) und mucin gene expression (qPCR).

Results

Phytochemical analyses of various freshly collected berries and blueberry preparations (dietary supplements) from the German market revealed considerable differences in quality:

I) **Freshly collected berries** from *V. myrtillus* showed the typical AC-pattern of reference solutions in TLC and HPLC. Cultivated blueberries from *V. corymbosum* had a reduced content of anthocyanidin-3-*O*-glucosides in HPLC analysis and this can be used as a discrimination marker to extracts obtained from *V. myrtillus*. Adulteration of bilberry extracts with cranberries or elderberries showed significantly different HPLC profiles.

II) Commercially available **dried bilberries** generally showed good quality. However, in one product, the declaration did not match the actual content due to production or storage-dependent degradation of AC. In three other products the tannin content of dried fruit was below the requirements specified in European Pharmacopoeia (Ph. Eur. 11.0) for dried blueberries.

III) **liquid** bilberry juices or nectar displayed the AC profile of the reference solution, although the nectar showed lower AC content. Eye drops containing bilberry extract and a homeopathic mother tincture did not contain any AC.

IV) Eleven **dietary supplements** examined showed serious quality problems. Besides seven commercial products with sufficient quality (however, in two cases with low AC content and in three cases with failed uniformity tests) four products were identified with low or absent AC content. In two of these samples, AC from black rice (*Oryza sativa* L. subsp. indica) was identified as a likely source of the AC in the product. Therefore, only 4 of 11 dietary supplements fulfilled all specifications concerning identity, content and mass uniformity. Fresh or dry fruits or juices should therefore be preferred by consumers.

Within *in vitro* analysis with human intestinal epithelial cell lines, the stability of bilberry AC in cell culture medium was very limited (half-lives between 17 and 45 min), which demonstrated the necessity of future systematic studies with AC and their degradation products.

Treatment of HT-29 cells with 250 µg/mL bilberry extract (BE) induced a significant increase (approximately 20 %) in the amount of mucin. An increased expression of genes related to mucin formation was not observed. Increased mucin secretion is discussed to strengthen the barrier function of the intestinal epithelium and which could have positive influence on inflammatory processes.

Discussion

The present study combines a detailed pharmaceutical analysis of AC in fresh/dried bilberries and liquid preparations or bilberry-containing dietary supplements with the use of BE *in vitro* model with investigations on two intestinal epithelial cell lines and thus contributes to a better understanding of the potential of these herbal preparations for the adjuvant treatment of IBD.