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Liquid-Liquid Chromatography: A versatile and scalable separation technique for the purification of minor hop compounds

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Solid support-free liquid-liquid chromatography, also known as countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC), is a highly selective, versatile and scalable separation technique for the isolation, purification and separation of active compounds from natural resources, biotech and biosynthetic products [1]. The separation is achieved as a result of the different partitioning of the mixture compounds between two phases of a multi-solvent biphasic system. One of the phases is kept in place with the help of a centrifugal force field, while the second one is pumped through.

In the past few years, natural phenolic plant compounds have shown promise due to their versatile bioactive potential. As an excellent source of a wide variety of such biologically active constituents, hop (*Humulus lupulus L.*) has been attracting scientific attention. Xanthohumol (XN) and other prenylated flavonoids belong to the most studied polyphenolic hop compounds [2]. Recent studies strongly emphasize their potential for the treatment of different diseases. Especially, some minor XN analogues were shown to exceed the bioactivity of XN itself [3, 4]. Existing methods for the isolation and purification of minor hop compounds are complex and inefficient. They involve several extraction and chromatographic steps, such as flash chromatography and preparative HPLC, and they suffer from low overall yield and irreversible adsorption [2]. Therefore, new strategies for the isolation and production of minor components from plant extracts in high purity as well as sufficient amounts are needed.

In the present study, CCC/CPC is demonstrated to be a promising technology for isolating and purifying Xanthohumol C, a minor hop compound, in sufficient amounts. Two different separation strategies for the purification of the minor hop compound Xanthohumol C were evaluated. The first method consisted of a two-step liquid-liquid chromatographic process, where a capture and enrichment step was followed by another CCC purification step. A second production strategy involved a one-step semi-synthesis starting from XN and a subsequent CCC/CPC purification. The second approach lead to higher Xanthohumol C purity and productivity, while simultaneously requiring less solvent. These methods promise high product recovery with low irreversible adsorption. The proposed methods thus provide a further step towards the exploitation of CCC/CPC as a highly flexible separation technique in downstream processing of active minor ingredients from crude extracts.

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