



Plant extracts in cell-based anti-inflammatory assays—Pitfalls and considerations related to removal of activity masking bulk components

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Plants used in traditional medicine represent an important source of new lead compounds. However, cell-based *in vitro* screening assays with plant material are hampered by the complex nature of plant extracts as mixtures of active and inactive components. Bulk constituents, such as chlorophyll and polyphenols were previously shown to interfere with several biological *in vitro* assays. Their influence on anti-inflammatory cell-based testing systems has not been thoroughly investigated. Hence, the present study was aimed at comparing different procedures for the removal of bulk constituents from plant extracts and examining the influence of their elimination on selected cell-based anti-inflammatory assays.

Malva sp. and *Glechoma hederacea* L., two plants used in traditional European medicine for the treatment of inflammatory disorders, were subjected to three different methods for the removal of chlorophyll and polyphenols, respectively.

Résumé en anglais

Removal of bulk constituents was confirmed by HPLC and mass spectrometry. Extracts were tested before and after the purification procedure, to determine their potential to inhibit the activation of the transcription factor NF- κ B in reporter gene assay and to interfere with the secretion of the chemokine IL-8 after stimulation of endothelial cells with tumor necrosis factor (TNF- α) or lipopolysaccharide (LPS). Removal of chlorophyll from tested extracts led to a strong decrease in the anti-inflammatory activities, due to loss of bioactive constituents. In contrast, the effect of the polyphenol-free extracts was either not changed or significantly increased, depending on the purification method used. The study concluded that clearance of bulk compounds represents a valuable strategy for cell-based *in vitro* anti-inflammatory evaluation of plant extracts. Liquid-liquid partitioning was identified as the optimal method for the elimination of both chlorophyll and polyphenols. It is recommended that removal of chlorophyll from extracts always be accompanied by HPLC profiling to detect a possible loss of active constituents.

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