



# Fishing and knockout of bioactive compounds using a combination of high-speed counter-current chromatography (HSCCC) and preparative HPLC for evaluating the holistic efficacy and interaction of the components of *Herba Epimedii*



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## ABSTRACT

Due to the complex chemical compositions and pharmacological effects of traditional Chinese medicines, we developed a strategy based on fishing and knockout of bioactive compounds using a combination of high-speed counter-current chromatography (HSCCC) and preparative HPLC for evaluating the holistic activity and interaction of the components of *Herba Epimedii*. First, osteoblast target cell extraction was used for preliminary screening of the potential bioactive compounds of *Herba Epimedii*. Second, the bioactive compounds identified (epimedin A, epimedin B, epimedin C and icariin) were fished and knocked out using high-speed counter-current chromatography and preparative HPLC. Third, the bioactivity of resulting fractions was assessed by determining their influence on cell proliferation and differentiation, thereby allowing for an evaluation of their interaction. The pharmacodynamic contribution ratio of each bioactive compound to the efficacy of the herbal medicine could then be comprehensively and intuitively determined based on the spectra-activity correlations (VIP values) of the tested compositions using partial least-squares regression (PLS-R), through which the reliability of the screening and isolation of bioactive compounds by the target cell extraction technique were verified. The proposed strategy is a useful approach with potential application in other traditional Chinese medicines.

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## 1. Introduction

*Herba Epimedii* is obtained from aerial parts of *Epimedium*, which includes many species, such as *Epimedium brevicornu* Maxim, *Epimedium pubescens* Maxim, *Epimedium sagittatum* (Sieb. et Zucc), *Epimedium wushanense* T.S. Ying and *Epimedium koreanum* Nakai. It is a famous herbal medicine that contains several medically active constituents, including flavonoids and phytosteroids, which are commonly used in China, India, Japan and Korea. According to traditional Chinese medicine (TCM) theory, *Herba*

*Epimedii* is classified as a 'kidney-tonifying' herbal medicine. It has been used extensively to treat osteoporosis, arthritis, impotence and lumbago for thousands of years (An et al., 2000; Liu et al., 2005). A direct relationship between kidney-tonifying functions and bone formation has been confirmed by accumulating evidence from studies demonstrating that *Herba Epimedii* has potential activity against osteoporosis (An et al., 2000; Ma et al., 2002; Zhang et al., 2008).

TCMs contain complex and multiple constituents that are responsible for their therapeutic effects through complicated synergistic and/or suppressive actions. Under the traditional separation and analysis model, which serves to identify new compounds, the success rates of obtaining highly bioactive natural products are generally lower due to the lack of bioactive markers. Recently, many screening techniques for evaluating bioactive compounds that focus on function fingerprinting, serum pharmacology and computer technology have been developed (Luo et al., 2008; Zhao et al., 2009; Zhai et al., 2012) and found to be effective because of their high throughput and high availability.

**Abbreviations:** HSCCC, High-speed counter-current chromatography; PLS-R, Partial least-squares regression; TCM, Traditional Chinese medicine; MTT, 3-(4, 5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; ALP, Alkaline phosphatase; DMSO, Dimethylsulfoxide; BCG, Bioactive-composition group.

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However, most of these methods overlook the relationships between the proportions of components and activity and cannot elucidate the interaction of multiple bioactive compounds. This highlights the importance of developing appropriate methodologies to reveal the correlations between the pharmacodynamic action of *Herba Epimedii* and its components.

In this study, we developed a strategy based on the fishing and knockout of bioactive compounds using a combination of HSCCC and preparative HPLC to evaluate the holistic activity and interaction of the constituents of *Herba Epimedii* (Fig. 1). First, a combination of specific target cells and HPLC techniques can be applied to the preliminary screening of the bioactive components of *Herba Epimedii*, which has the advantages of high separation capacity and accurate quantitation under the premise of maintenance of the normal physiological state of the cell (Zhao et al., 2000; Lin et al.,

2006). Second, selective fishing and knockout of the main bioactive components of *Herba Epimedii* were performed using HSCCC and preparative HPLC. Third, the efficacies of the compounds obtained by fishing and knockout technology were evaluated for anti-osteoporotic bioactivity, which could be estimated by determining the influence of each compound on the proliferation and differentiation of rat calvarial osteoblasts using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and by measuring the activity of alkaline phosphatase (ALP) (Wang et al., 2002; Meng et al., 2005).

By comparing not only the proliferation promotion ratios and differentiation inhibition ratios of the multiple bioactive constituents with the compounds that they knocked out but also the summation proliferation promotion ratio and differentiation inhibition ratio of several bioactive constituents with those of total

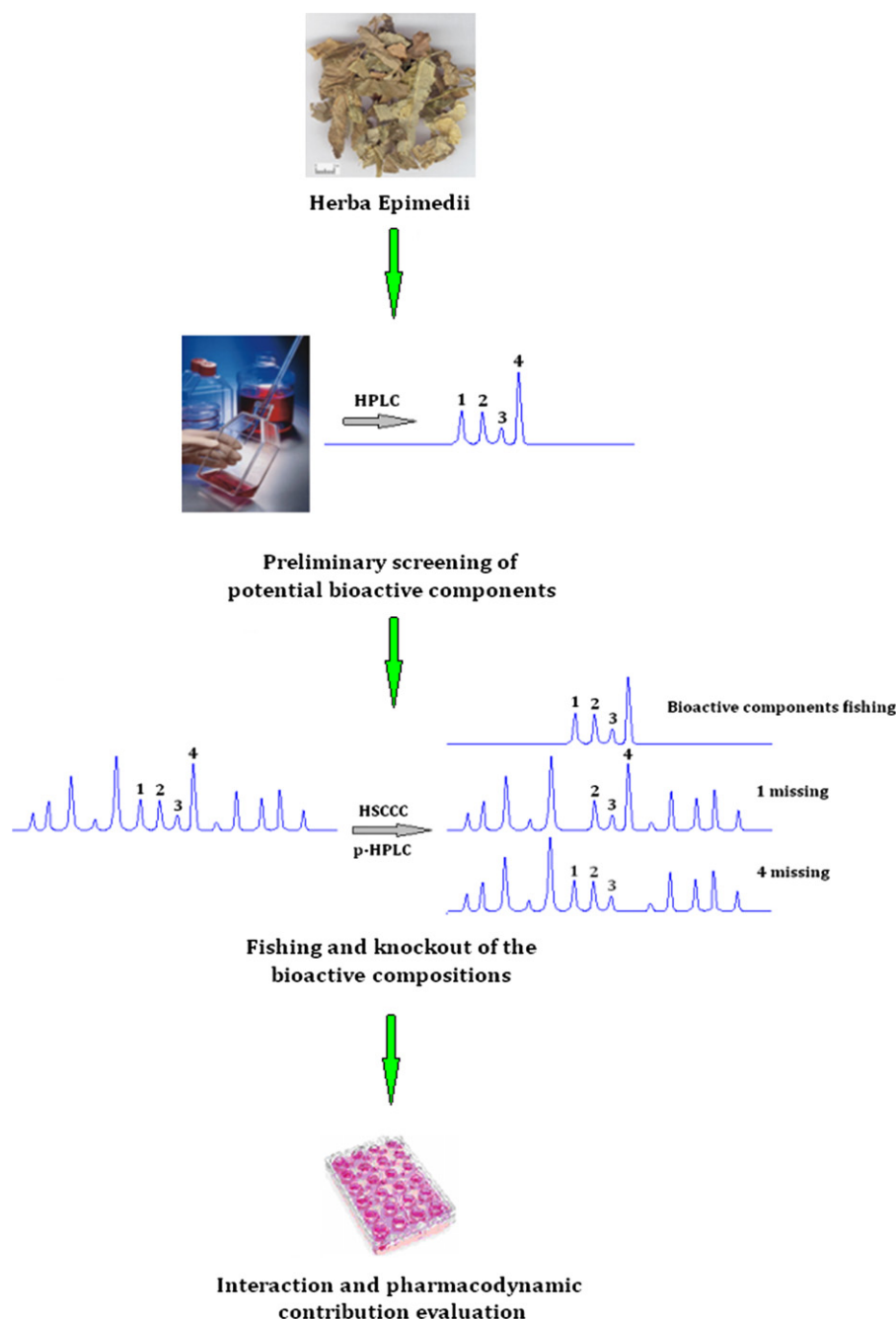


Fig. 1. Schematic diagram representing the strategy for holistic efficacy and interaction evaluation of 2 of *Herba Epimedii*.

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