

Short Communication

Selective extraction of berberine and palmatine from Huangbai powder

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Abstract: Berberine and palmatine are two of the main bioactive components in Huangbai, a major Chinese medicinal herb. The current methods to extract these compounds usually involving the usage of inorganic acid and base, are not only complex and time-consuming, but have a low selectivity. In this paper, it was reported that hexane, ethyl acetate and dichloromethane were tested to extract berberine and palmatine from Huangbai powder. The results showed that dichloromethane extracted selectively and effectively berberine and palmatine from Huangbai powder among the examined solvents. In addition, dichloromethane can be recycled and reused, making it a potential candidate for large scale extraction of berberine and palmatine from Huangbai.

Keywords: Berberine, dichloromethane, palmatine, huangbai powder, selective extraction, simple and effective separation

INTRODUCTION

Natural products have been and will be important sources of new pharmaceutical compounds (Rouhi and Washington, 2003). Usually, it is difficult to extract and purify targets from plants, animals and other medicinal resources, which is attributed to chemical structural diversity and the intricacies of natural products in these medicinal resources as well as low content of targets. However, the development of modern pharmaceutical industry pursues a simple and effective strategy to extract these targets (Lang and Wai, 2001). The pursuit of these extract targets with increasing complexity has resulted in the development of extraction methods that emphasize selectivity (Lee *et al.*, 2009). In using strategies to extract targets from an intricate defining system, selectivity is mandatory (Choi *et al.*, 1999).

Huangbai (*Cortex Phellodendri*) is one of the most important traditional Chinese medical herbs (Tsai *et al.*, 2004). It is categorized as an internal heat relief medicine and is effective in curing dysentery, diarrhea, and other syndromes (Xiao, 1997). In regard to its impact on the alimentary tract, it seems to exert a totally different affect on the movement of water across the intestinal epithelia.

Berberine and palmatine are two of the main bioactive components in Huangbai (Birdsall and Kelly, 1997). Various additional pharmacological effects of berberine have been also reported, such as anti-tumour (Iizuka *et al.*, 2000), anti-inflammation (Ckless *et al.*, 1995), anti-coagulation (Mahajan *et al.*, 1982), and hypotensive effects (Chun *et al.*, 1979) and inhibition of ventricular

tachyarrhythmia (Chun *et al.*, 1979). Also, palmatine has been shown to have similar pharmacological effects (Park *et al.*, 1999). Therefore, efforts have been devoted to develop an effective method for their extraction from Huangbai (Ying *et al.*, 2007; Yang *et al.*, 2002; Han *et al.*, 2000; Gong *et al.*, 1998; Chu *et al.*, 2000). However, the current methods are often not only complex and time-consuming, but have a low selectivity. Here we report on a method that has been developed to selectively extract berberine and palmatine from a water extract of Huangbai powder.

MATERIALS AND METHODS

Materials

Huangbai powder was purchased from Bozhou City Houpu Pharmaceutical Co., Ltd., China. Hexane, ethyl acetate, dichloromethane, acetonitrile and phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai. All chemical reagents were analytical grade.

Extraction

To 10/g Huangbai powder, 20/ml hexane was added 4 times. Each time, the mixture was fully stirred until the distribution of components in two phases was equilibrated, and then filtered. The organic phase was combined. Then the combined organic phase was concentrated by a rotator. The resulting fraction was determined by high performance liquid chromatography (HPLC).

In addition, methanol was used as solvent to obtain HPLC profile. To examine the efficiency, methanol was also used as solvent to extract the residue of Huangbai powder extracted with dichloromethane.

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HPLC analysis

The extract was analyzed using HPLC under a wavelength of 254 nm. All analyses were performed on a Waters 2695 high pressure liquid chromatograph equipped with a HT300L auto sampler coupled with a UV spectrometer. Agilent Zorbax SB-C18 (1.5/μm 4.6×150 mm) was used. The oven was held at 25°C. The mobile phase comprised of acetonitrile and phosphate (1.5/ml concentrated H₃PO₄ per 1000/ml H₂O) and the ratio of acetonitrile 2% at 0 min, 25% at 45 min, 35% at 70 min, 55% at 85 min and 75% from 95 to 100 min.

RESULTS

Fig. 1 shows the HPLC fingerprint for Huangbai powder. Here, methanol was used as solvent. From fig. 1, it was determined that the retention times of berberine and palmatine (peak 10 and 9) were different from those of all other chemical components.

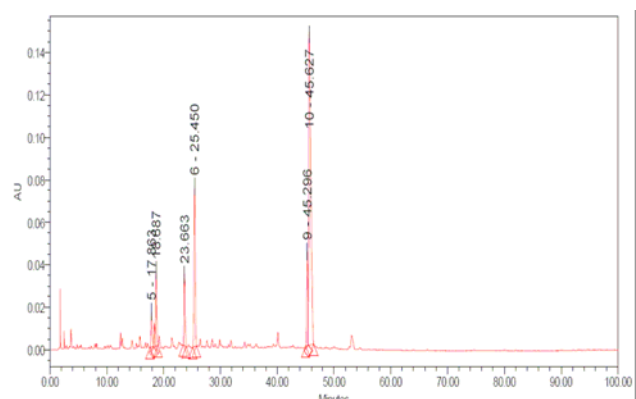


Fig. 1: HPLC fingerprint for Huangbai powder. Methanol was used as solvent

Subsequently, a variety of organic solvents were tested for this purpose. The results are illustrated in fig. 2-4. Fig. 2 and 3 showed the HPLC results of the fractions extracted with ethyl acetate and dichloromethane, respectively. Fig. 4 shows the HPLC result of the residue of Huangbai powder that was extracted with dichloromethane.

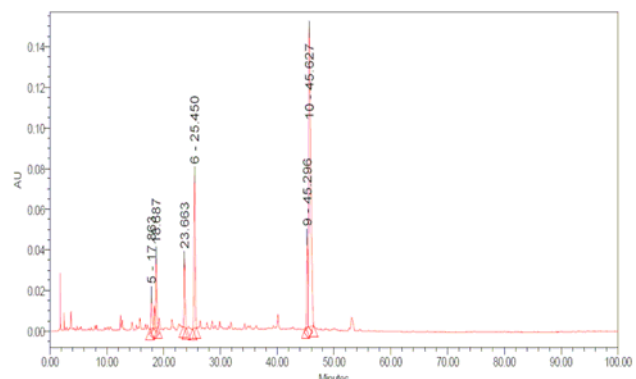


Fig. 2: HPLC result for fraction of Huangbai extracted with ethyl acetate.

First, hexane was tested (not shown). Some non UV-active components were extracted from Huangbai powder when using hexane. Components that had relative high polarity remained with the residue.

Therefore, a high polar solvent, ethyl acetate, was examined. The result is illustrated in fig. 2. The ethyl acetate-extracted fraction contained not only berberine and palmatine, but other chemical components as well. It was speculated that a solvent with a polarity ranging between hexane and ethyl acetate would meet the requirement. Thus, dichloromethane was tested and the result is shown in fig. 3. From fig. 3, it was determined that berberine and palmatine were the main composition in the dichloromethane-extracted fraction.

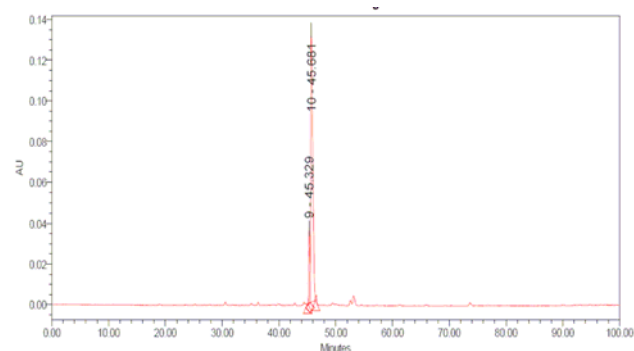


Fig. 3: HPLC result for fraction of Huangbai extracted with dichloromethane.

To examine the efficiency, the residue of Huangbai powder extracted with dichloromethane was extracted with methanol, and then monitored by HPLC. The result is shown in fig. 4. As shown in fig. 4, the peaks standing for berberine and palmatine respectively were very weak, which showed that both berberine and palmatine were separated completely from Huangbai powder.

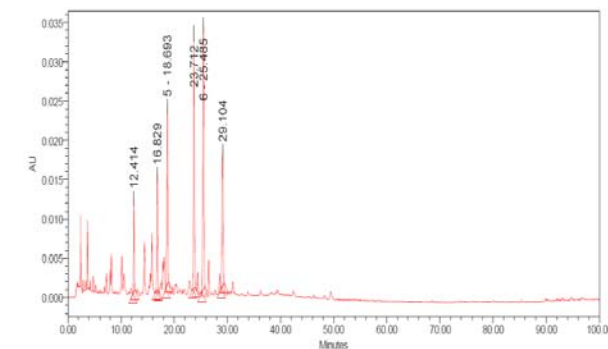


Fig. 4: HPLC result for the residue of Huangbai extracted with dichloromethane. Methanol was used as solvent

DISCUSSION

The retention times of berberine and palmatine (peak 10 and 9) were different from those of all other chemical

components of Huangbai powder. Theoretically, the results showed that it was possible to find a suitable solvent for separating them from other components in Huangbai powder. Among the examined solvents, the polarity of hexane was too weak to extract effectively berberine and palmatine, while that of ethyl acetate was too strong to extract selectively berberine and palmatine. Dichloromethane was not only effective but selective for separating berberine and palmatine from other chemical components in Huangbai powder. Moreover, although a few other chemical compositions existed, the dichloromethane-extracted fraction was pure enough to crystallize for further purification purposes. An added benefit, dichloromethane can be recycled and reused during this process, thus keeping costs relatively lower, and the process did not involve other organic solvents, acidic or base media. Therefore, the method reported here is simple, effective, and cost-effective.

CONCLUSION

We show that dichloromethane selectively extracts berberine and palmatine from Huangbai powder. The method proposed here is simple and effective. Therefore, it is a promising alternative to the present method for the separation of berberine and palmatine from Huangbai and further research is in progress.

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