Ultrasound-assisted solvent extraction of swainsonine from *Oxytropis ochrocephala* Bunge

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Swainsonine is an indolizidine alkaloid with anticancer and antiviral potency. Extracting from locoweeds is one of strategies to produce swainsonine currently. Here, ultrasound-assisted solvent extraction was reported to significantly promote isolation and purification of swainsonine from *Oxytropis ochrocephala* Bunge, a popular locoweed in northwestern China. The extracted swainsonine was identified by thin layer chromatography, melting point, gas chromatographic and mass spectrometric analysis. The straightforward and simple method described here would make swainsonine more readily available and aid in medical studies.

**Key words:** Swainsonine, ultrasound, solvent extraction, *Oxytropis ochrocephala* Bunge.

**INTRODUCTION**

Locoweed is a common name for those species of the genera *Astragalus* and *Oxytropis* (family Leguminosae) that produce swainsonine (Figure 1). After grazing locoweeds, animals often develop locoism with neurological changes, reproductive disturbance, and emaciation. Toxicology, pathology, and grazing aspects of locoweeds have been reviewed extensively (Panter et al., 1999; Ralphs and James, 1999; Stegelmeier et al., 1999) and swainsonine is identified to be the murderer. However, swainsonine was reported recently to be an anti-cancer drug with potential for treating glioma (Sun et al., 2009) and gastric carcinoma (Sun et al., 2007). Antiviral effects of swainsonine on respiratory syncytial virus were also reported (McDonald et al., 2006). Many studies indicate that swainsonine would be a promising compound with medical significance.

Presently, swainsonine is mainly extracted from locoweeds or fungi (*Rhizoctonia leguminicola* or *Metarrhizium anisopliae*). It also can be produced from total synthesis. The use of locoweeds as a source material for swainsonine is attractive because large amounts of plant material can be collected, dried and stored, as opposed to the maintenance of batch fungal cultures (Gardner et al., 2003). Although, swainsonine content in locoweeds varied geographically and taxonomically, those with the highest alkaloid content could be selected for extraction. The *Oxytropis* locoweed species distribute wildly and provide an excellent extraction source of swainsonine. Traditional Soxhlet extraction or solvent extraction is currently used for isolating swainsonine from locoweeds (Gardner et al., 2001). The main defect of these methods is low efficiency. In this study, we reported that ultrasound-assisted solvent extraction significantly promoted isolation and purification of swainsonine from *Oxytropis ochrocephala* Bunge. Such a relatively straightforward and simple method would make swainsonine more readily available and aid in medical studies.

**MATERIALS AND METHODS**

**Plant materials**

*O. ochrocephala* Bunge (One species of locoweed) was collected by clipping the plant stems just above the ground from HaiYuan County (Ningxia Province, China) in October 2008. Taxonomical identification of the collected plants was made by Prof. Langran Xu (College of Plant protection botany, Northwest A and F University). The aerial plant materials, including flowers, stems and leaves, were made into powder and used for swainsonine extraction.

**Swainsonine extraction**

One kilogramme *O. ochrocephala* bunge powders were pretreated
in 10 L water at 60°C with or without ultrasound for 1 h. Crude extraction was concentrated to 2 L and then centrifuged (1500 r/min) for 10 min to remove plant materials. The upper solution was further concentrated to 1 L followed by extraction with n-butanol extracting, sulphuric acid extracting, ammoniated chloroform extracting and subliming. The experimental route was shown in Figure 2.

**Swainsonine identification**

The purity of the extracted swainsonine was determined by thin layer chromatography as described previously (Davis et al., 1984) with modification. Briefly, 0.5 mg white crystals were dissolved in 100 µL methanol and developed on silica gel plates in CHCl₃:CH₃OH:NH₄OH·H₂O (70:26:2:2). The plates were stained with iodine steam or Ehrlich reagent. Standard swainsonine (Sigma Cat. 86204) was used for positive control. The melting point was measured by X-6 micro melting apparatus.

Gas chromatographic analysis was accomplished as our previous study (Zhao et al., 2009) with minor modifications. Briefly, 0.1 mg white crystals were dissolved in 40 µL of pyridine and well mixed with 40 µL of the internal standard (me-Gal) at a concentration of 0.25 mg/ml, and 20 µL of N, O-bis(trimethylsilyl)
trifluoroacetamide + trimethylchlorosilane (BSTFA+TMCS). After derivatization for 30 min at room temperature, the mixture (1 µL) was injected for the gas chromatographic analysis. Shimadzu model 14C gas chromatograph equipped with flame ionization detector (FID) and AT.SE-54 column were used in this study. The column temperature, injector port temperature and detector block temperature were 210, 280 and 300°C, respectively. Purified dry column temperature, injector port temperature and detector block were 210, 280 and 300°C, respectively. Purified samples of swainsonine (white crystals) were further analyzed by Mass Spectrometer. The mass detector was operated at electron-impact mode (70 eV) with source temperature at 230°C.

Statistical analysis

The experiments were performed in triplicate and data were analyzed by one-way analysis of variance (ANOVA) and multiple comparisons. Mean values and standard deviations were calculated on software of the SPSS and presented.

RESULTS AND DISCUSSION

Extraction of swainsonine

O. ochrocephala Bunge is one species of Oxytropis plants, which distributes extensively on grasslands in northwestern China (Zhao et al., 2003). The aerial parts of O. ochrocephala Bunge were collected, dried and made into powder for swainsonine extraction. Although, application of ultrasound has been reported to promote extraction of water soluble polysaccharide (Hromadkova et al., 1999), oil (Li et al., 2004), and other biologically active substances (Semagina et al., 2000), no investigation of ultrasound on extracting swainsonine has been made so far. In this study, pretreatments with or without ultrasound were compared (Figure 3). The results showed that yield of white crystals (swainsonine sample) from 50 kHz ultrasonic treatment was significantly higher than others. It was speculated that 20 kHz ultrasonic treatment was not enough to destroy the plant cells and release swainsonine. However, high frequency of ultrasound (80 kHz) may cause local extremes of temperature and pressure which destroyed the structure of swainsonine. The mechanism under ultrasound-assisted solvent extraction of swainsonine needs further investigation.

Characterization of swainsonine

The purity of the white crystals was determined by thin layer chromatography. It can be seen that the purified material had the same migration rate as the authentic swainsonine, visualized with either Ehrlich's reagent or iodine steam (Figure 4). The melting point of purified white crystals was also observed. The white crystals had melting point of 144~145°C, which was identical to the available standard swainsonine from Sigma. The white crystals were also identified by gas chromatography. Retention time of both white crystals and authentic swainsonine was 5.45 min (Figure 5). The purified material was further compared to authentic swainsonine by mass spectrum as shown in Figure 6, which revealed that the retention time, overall fragmentation pattern and percentage relative intensity were the same for both samples. All the data indicated that white crystals were swainsonine with high purity.

Conclusion

Ultrasound-assisted solvent extraction was a reliable,
Figure 4. Thin layer chromatographic identification of extracted swainsonine. Staining was visualized with either iodine steam (A) or Ehrlich's reagent (B). A1, B1 indicates authentic swainsonine, A2, B2 indicates extracted white crystals. $R_f = 0.44$.

Figure 5. Gas chromatograph of swainsonine. Authentic swainsonine (A) and extracted swainsonine (B) were compared.
simple and effective method for fast isolation and purification of swainsonine from *O. ochrocephala* Bunge. The extracted swainsonine was identified by thin layer chromatography, melting point, gas chromatographic analysis and mass spectrometric analysis.

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REFERENCES