A Novel Purification Process for Homoharringtonine from *Celphalotaxus koreana*

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ABSTRACT

An effective purification method was developed for producing Homoharringtonine (HHT), to guarantee high purity and yield from *Cephalotaxus koreana*. This process was a simple and efficient procedure, for the isolation and purification of HHT form the biomass of *Celphalotaxus koreana*, consisting of extract, adsorbent treatment, precipitation and followed by a chromatography. The extraction, adsorbent treatment and precipitation in pre-purification process allows for rapid and efficient separation of HHT from many compound and dramatically increases the yield and purity of crude HHT for HPLC purification steps compared to alternative processes. This purification processes serves to minimize solvent usage, size, and complexity of the operations for HHT purification.

INTRODUCTION

Homoharringtonine (HHT), an alkaloid isolated from genus *Cephalotaxus*, is an alkyl substituted succinic acid ester of cephalotaxine (Fig.1). It possesses antileukemic activity and is a potent myelosuppressive agent. Recent studies suggest that HHT inhibits tumor growth by inducing apoptosis. Its potent antileukemic activity and limited toxicity have prompted the National Cancer Institute (NCI) to develop HHT as antitumor agent. Through synthesis of cephalotaxine and its esters has been reported, extraction from plants is still the major source for HHT. The present work attempts to develop a new purification method for mass production of HHT from *Cephalotaxus koreana*. This process is a simple and efficient procedure, for the isolation and

purification of HHT with a high purity and yield. This purification process serves to minimize solvent usage and the size and complexity of the HPLC operations for HHT purification.

MATERIALS AND METHODS

Plant materials for extraction

Plant materials was used a stem, a twig, and a leaf from *Cephalotaxus koreana*. These were drying for 16 hours at 60℃, then crushing, and making powders through 0.1mm mesh.

Analysis of HHT

HPLC was used for all analytical characterizations of intermediate or finished products. HPLC assay was performed with C_{18} column (shiseido,4.6×250mm,5㎛). The mobile-phase was used methanol and 0.1M ammonium formate solution. Elution was performed by gradient condition with a mixture of methanol and 0.1M ammonium formate began at 20:80(v/v) to 80:20(v/v), after 30min gradient condition of solvent was became 40:60(v/v) to 60:40(v/v) (flow rate=1.0 ml/min). Detection was by UV at 290nm. Injection volumes were 20㎕. Quantity of Homoharringtonine was calculated peak area of standard material(sigma, purity=98.6%), after drew up standard quantitative-line.

RESULTS AND DISCUSSION

Solvent extraction

Several solvents or combinations of solvents were tested for the extraction of biomass. Of these, 90% ethanol gave the highest HHT recovery with the least amount of solvent usage and was therefore chosen for all subsequent process development work. After three-times extraction of biomass with 90% ethanol, the concentration to obtain aqueous residue of the 90% ethanol extract was accomplished by rotary evaporators at vacuum of 40℃. At the using solvent for extraction, recovery of more 99% obtained by three-times 90% ethanol extraction from biomass (Fig.2). Then

three-times liquid-liquid extraction of equal quantity from chloroform, the concentration of the pH5 of chloroform-phase was accomplished by rotary evaporators at a vacuum of 40℃ (Fig.3). After powders dissolved in methanol (1ml/g), analyzed by HPLC through the filtration with 0.2㎛ filter. Polar impurities were removed by liquid-liquid extraction process.

Adsorbent treatment

Since the impurities, such as waxy substances, in the dried crude extract obtained in the 90% ethanol extraction step played an obstructive role in subsequent purification steps, synthetic adsorbents were added to remove the waxy substances. The synthetic adsorbents used were active clay. Active clay was also more effective in the removal of impurities and for obtained a high yield at using methylene chloride rather than chloroform.

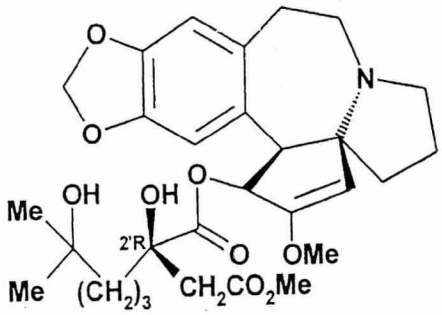

Figure. 1 Structure of HHT.

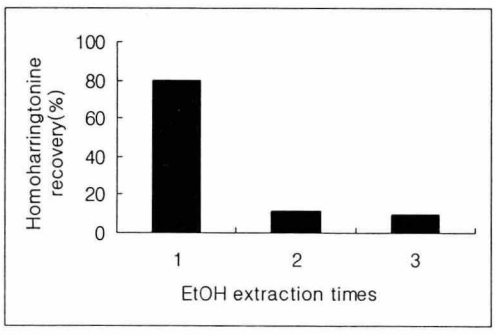

Figure. 2 Effect of ethanol extraction timeson HHT recovery from biomass.

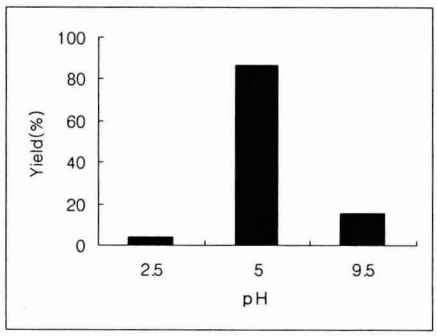

Figure. 3 Effect of pH on yield in liquid-liquid extraction.

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