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*Research Article*

# Components and Anti-HepG2 Activity Comparison of *Lycopodium* Alkaloids from Four Geographic Origins

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*Lycopodium japonicum* Thunb. has attracted great interests due to its rich alkaloids with significant anticancer activity. However, significant chemical differences often exist in a plant species from different geographic origins and affect its quality and bioactivities. Thus, it is urgent to reveal their chemical and biological distinctions at the molecular level. In this context, a comparative chemical analysis of LAs using HPLC-UV-ESI-MS/MS was firstly conducted and resulted in the detection of 46 LAs, 28 of which were identified, and a series of unique LAs markers, such as peaks **2**, **9**, **10**, and **11**, were further found to be characteristic LAs and selected as markers from four different origins for their quality control. In parallel, the comparative bioactivity assay revealed that the total LAs from Hubei province exhibited much higher inhibitory rate at 65.95% against HepG2 cells than those at 26.72%, 20.26%, and 33.62% for Kenya, Guangxi province, and Zhejiang province in China, respectively. To this end, significant chemical fingerprinting differences and discrepancies in bioactivity of LAs were explored firstly, which could provide valuable information for quality control and further activity studies on LAs from different sources and promote their better pharmaceutical applications in the future as well.

## 1. Introduction

*Lycopodium japonicum* Thunb is a traditional medicinal herb in China, which has been used for the treatment of a variety of diseases for thousands of years, such as contusion, analgesia, and rheumatoid arthritis [1, 2]. It has been reported that lycopodium alkaloids (LAs) were the major bioactive components widely found in the plants of *Lycopodium* genus, for example, *L. obscurum* [3], *L. annotinum* [4], and *L. chinense* [5]. Since these LAs have been proved to possess a wide spectrum of bioactivities, for example, anti-inflammation, antitumor, and acetylcholineasterase inhibitory activity [6–9], many efforts focusing on the isolation, synthesis, identification, and biogenetic synthesis of LAs have been made to explore and expand this valuable medicinal resource [10]. To date, up to 300 LAs were reported mainly from various *Lycopodium* and *Huperzia* genus plants or from one plant with different growing stages and environments [11]. Among them, geoorigin of a plant is an important factor affecting the types and chemical structures of LAs since they are produced through plant metabolism and its complex interactions with the growing environments. Therefore, comparative study on the LAs of Lycopodium genus plants from different areas can provide valuable information on the evaluation of their chemical similarities and differences and further their pharmaceutical activity discrepancies.

To investigate LAs from a plant species, the traditional phytochemical approaches usually involved multistep isolation and structural elucidation of pure compounds from a plant of interest, which are often tedious, complex, and time-consuming [3]. Because of the excellent performance on simultaneous separation and identification of multicomponent mixtures with complex background, chromatography based separation techniques (i.e., GC and LC) coupled with various detectors, such as mass spectrometry (MS), were developed as useful tools in most cases [12]. Since most LAs