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Effects of growth altitude on chemical constituents and delayed luminescence properties in medicinal rhubarb

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ABSTRACT

To improve the quality control of herbal drugs, there has been a major shift from evaluating individual chemicals to evaluating multiple-constituent chemicals, given the multi-pharmacology nature of herbal drugs. Therefore, rapid, systematic assays are needed in order to assess the quality of medicinal herbs using a comprehensive, integrated approach. Light-induced delayed luminescence (DL) is used to measure decaying long-term ultra-weak photon emissions following excitation with light. DL is considered to be a sensitive indicator for characterizing the properties of biological systems and herbal medicines with various therapeutic properties. The aim of this study was to examine the feasibility of using DL as a novel quality-assessment tool using rhubarb material as a model system, and to establish the correlation between DL parameters and the chemical constituents of rhubarb. Raw roots and rhizomes were collected from rhubarb (*Rheum palmatum* L.) at various elevations in western China. HPLC analysis was used to identify fourteen bioactive constituents. Five DL parameters were calculated from the DL decay curves of the rhubarb samples. Statistical tools, including principal component analysis, were used to classify the rhubarb samples using data obtained using two different assays. Finally, Spearman's correlation coefficient was calculated to quantify the correlation between the bioactive compounds and corresponding DL parameters. We found that both the chemical analysis and DL measurements reflect variations in the quality of rhubarb due to environment factor. The DL parameters were correlated significantly with the bioactive chemical constituents. Our results indicate that DL is a promising tool for evaluating multiple constituents and for assessing the therapeutic properties of herbal medicines. Thus, DL may be used as part of a comprehensive system for assessing the quality and/or therapeutic properties of herbal medicines.

1. Introduction

Environmental factors related to growth conditions are closely associated with the quality and therefore therapeutic properties of medicinal plants [1,2]. Since ancient times in China, medicinal plants have been produced and/or collected in specific geographic regions with unique ecological conditions; these so-called "indigenous medicinal materials" are used to represent the optimal quality and therapeutic properties of Chinese herbal drugs based on clinical practices and experiences [1]. It is generally accepted that secondary metabolites are important pharmacologically active constituents in medicinal plants [3]. Plants produce secondary metabolites as a means to adapt to their growth conditions, for example to provide protection from environmental stressors [2]. In addition, changes in environmental factors can be reflected in the accumulation of secondary metabolites [4]. Therefore, focus should be shifted toward controlling environmental conditions in order to manage quality and standardize herbal drugs [5].

Rhubarb (scientific name: *Rhei Rhizoma*) is an herbal drug that has been widely used for thousands of years throughout China [6,7]. The dried roots and rhizomes of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., and *Rheum officinale* Bail. are officially included in the

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Chinese Pharmacopoeia [8] and European community monograph [9]. According to the principles of traditional Chinese medicine, rhubarb is a typical herbal drug with many therapeutic properties, including catharsis, heat-clearing effects, detoxification, and removal of blood stasis [8]. In recent decades, chemical and pharmacological studies have shown that the pharmacological activities of secondary metabolites in rhubarb—including anthraquinone derivatives and polyphenol constituents—correspond with the traditional therapeutic functions associated with rhubarb (Fig 1) [10–14]. For example, rhubarb's detoxification property is reflected largely by its antibacterial activity, which is related primarily to free anthraquinones such as rhein, emodin, and aloe-emodin [10]. Rhubarb's cathartic property is due to the presence of both anthraquinone glycosides and sennosides [10,11,13]. Rhubarb's blood stasis–relieving properties have been attributed to (+)-catechin [14]. In addition, gallic acid has been proposed to have antiplatelet activity [15], which may also explain rhubarb's blood stasis–relieving properties. Moreover, additional pharmacological effects have been attributed to compounds isolated from rhubarb, including anti-cancer activity [16], anti-inflammatory activity [17], antioxidant activity [18], liver-protective properties [19], and improved renal dysfunction [20].

Given these important therapeutic properties, rhubarb has become one of most commonly used herbal medicines, and the demand for rhubarb has grown both in China and in global markets [11]. However, rhubarb is produced primarily in a limited region in western China at an altitude of 1000–5000 m [21], and overharvesting has caused a significant decline in rhubarb crops; in addition, overharvesting also causes considerable damage to the growing environment, causing variation in the quality of rhubarb grown [11]. Therefore, developing an effective tool for measuring quality control in rhubarb is very important. Given the high number of bioactive constituents in rhubarb and their high number of therapeutic properties, comprehensive, systematic methods for analyzing rhubarb quality are needed [11].

Delayed luminescence (DL) is long-term decaying, weak photon emission from various materials following exposure to light with a wavelength of 400–800 nm [22–25]. DL has been used as a tool for directly and rapidly assessing biological systems and has been found to provide a sensitive indicator of food quality [24–26]. Recent studies examined the DL signatures of dry powders prepared from Chinese herbal medicines [27,28]. These studies demonstrate that distinct DL properties can be measured between the same herbal medicines prepared under different conditions, including the age of the herb, environmental factors, and the processing method [27]. These differences in DL properties reflect variations in both the bioactive constituents contained in the herb and the therapeutic properties of herbal drugs [29–32]. Importantly, DL can be used to predict the herb's energetically therapeutic properties (i.e., "Cold" or "Heat") based on the principles of Chinese medicine

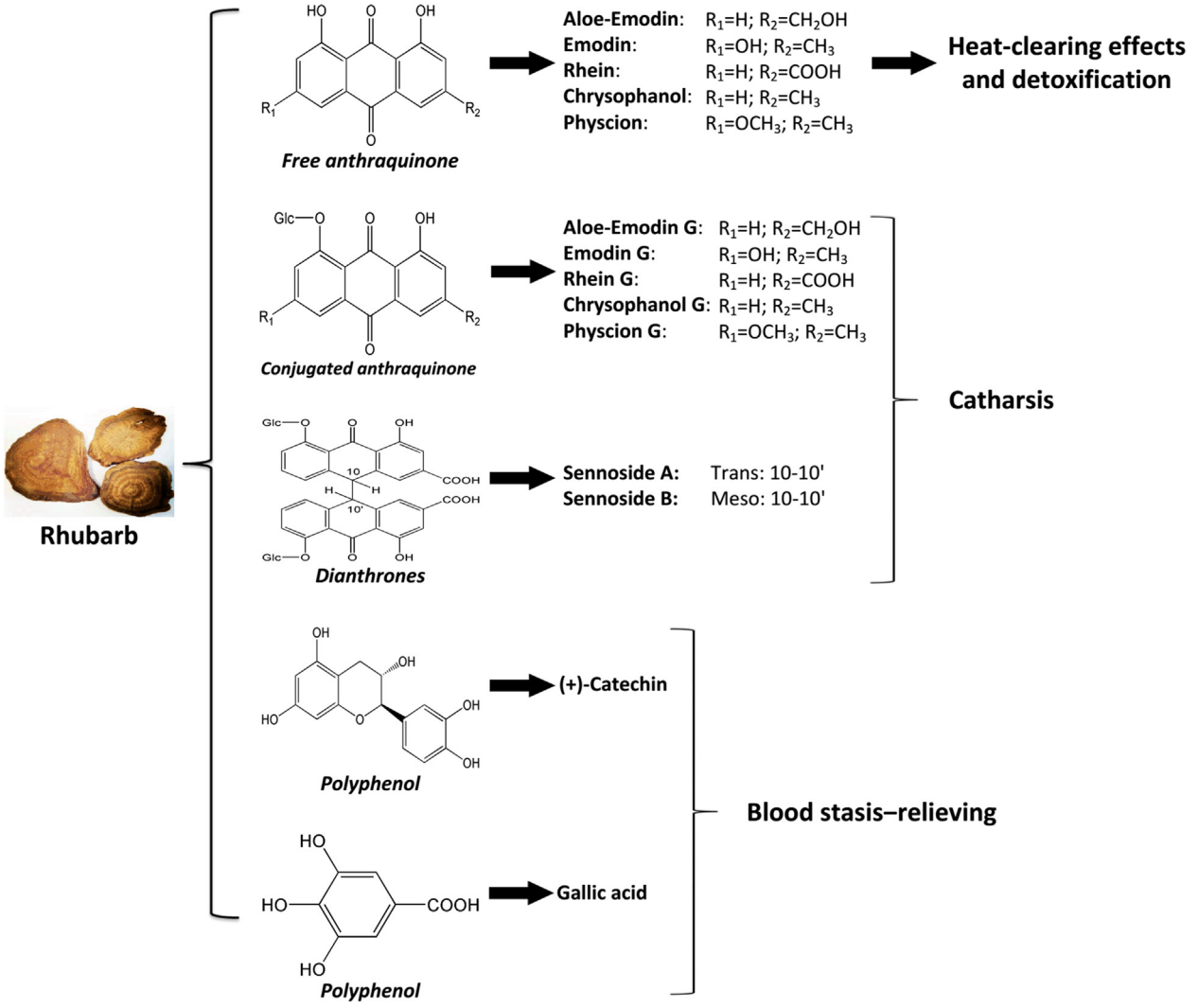

Fig 1. Schematic diagram of the chemical constituents in rhubarb and their role in Chinese medicine-based concepts. Fourteen chemical constituents in rhubarb correspond to various therapeutic properties in the principle of Chinese medicine. The terms in italics under the chemical structure indicate the structural characteristics of the chemical constituents. "Glc" in the chemical structure indicates a glycoside. "G" in the names of chemical constituents indicate glycosides. Conjugated anthraquinone represents anthraquinone glycosides with O-glycosides, where the aglycone moiety is an 8-dihydroxyanthraquinone derivative. Additional structures of conjugated anthraquinones not shown here are published elsewhere [11].