Spectral Analysis of Chinese Medicinal Herbs Based on Delayed Luminescence

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Traditional Chinese medicine (TCM) plays a critical role in healthcare; however, it lacks scientific evidence to support the multidimensional therapeutic effects. These effects are based on experience, and, to date, there is no advanced tool to evaluate these experience-based effects. In the current study, Chinese herbal materials classified with different cold and heat therapeutic properties, based on Chinese medicine principles, were investigated using spectral distribution, as well as the decay probability distribution based on delayed luminescence (DL). A detection system based on ultraweak biophoton emission was developed to determine the DL decay kinetics of the cold and heat properties of Chinese herbal materials. We constructed a mathematical model to fit the experimental data and characterize the properties of Chinese medicinal herbs with different parameters. The results demonstrated that this method has good reproducibility. Moreover, there is a significant difference \( p < 0.05 \) in the spectral distribution and the decay probability distribution of Chinese herbal materials with cold and heat properties. This approach takes advantage of the comprehensive nature of DL compared with more reductionist approaches and is more consistent with TCM principles, in which the core comprises holistic views.

1. Introduction

The cold and heat properties of Chinese medicinal herbs are classified according to the traditional Chinese medicine (TCM) principles, which comprise a core concept of TCM. The cold or heat property reflects a trend that Chinese medicinal herbs affect the transformation of heat or cold properties of the human body. Cold is a Yin disease factor that causes symptoms such as chilliness, headache, and body aches. Cold is reported to damage the Yang energy. Heat (also heat or flame) is also a Yang pathogenic factor with symptoms that include fever, inflammation, dry skin, and constipation. Chinese medicinal herbs regulate the cold or heat properties of the body to achieve a balance between them [1]. Cold herbs treat heat diseases, and heat herbs treat cold diseases. Thus, the cold or heat properties of Chinese medicinal herbs and the application of the corresponding knowledge regarding the diagnosis, differentiation, and treatment of diseases comprise important aspects in TCM. Recently, many groups have investigated herbs to identify their essence and properties [2–5]. However, the scientific evidence regarding the cold and heat properties of Chinese medicinal herbs has remained unclear. The development of a novel scientific method that provides a quantitative measure of these properties is a challenge and represents the aim of the present study.

Delayed luminescence (DL) is the long-term decay of ultraweak photon emission from biological systems following
exposure to illumination, and it was discovered in green plants in 1951 [6]. DL technology has recently been used as a noninvasive tool to investigate germination, food quality, tumor cells, and environmental pollution [7–11]. DL is correlated with the functional state of the biological sample [12–15], which suggests that DL measurements may represent a potentially valuable method to analyze the cold and heat properties of Chinese medicinal herbs [16].

Despite the broad utility of DL, no study has investigated the DL signatures of Chinese herbal materials. In this study, a sensitive photon-counting system was constructed to collect DL spectra and characterize Chinese medicinal herbs [17].

In this study, we report DL data collected from the roots of Chinese medicinal herbs. The aims are to identify a correlation between the physical parameters connected to DL and the cold and heat properties of Chinese medicinal herbs as well as establish an evaluation method and indicator of the cold or heat property of Chinese medicinal herbs. This approach provides a comprehensive picture of the herbs versus the more reductionist ideas used in chromatography approaches and is more consistent with the holistic view at the heart of TCM.

2. Material and Methods

2.1. Measurement System and Measurement Procedure of DL. The device (Figure 1) includes a dark sample chamber and a vertical photomultiplier tube (PMT) with a 46 mm photocathode (Electron Tubes Enterprises Ltd., UK, type 9558QB) [17]. The PMT is cooled to −25 °C to reduce the dark count rate to less than 10 counts per second.

A 55 mm Petri dish filled with the dried samples was placed in the dark chamber 12 cm from the PMT shutter. Herbs (1 g) covered the bottom of the Petri dish. To excite the samples, we used a white LED (LED Engin, USA, type LZ4-00MD00). The interval time was 100 ms. The measurement time was 20 s. A shutter system between the excitation source and the sample controlled the excitation. A photon-counting unit (HAMAMATSU, C9744) was used for all data acquisition.

During the DL measurement, a spectral analysis was performed with seven long-pass cutoff optical interference filters (Schott, Germany) [18, 19]. These filters were placed in a rotating wheel, which was located between the photomultiplier and the shutter in front of the photomultiplier. The rotating wheel has 8 openings: open (without filter), GG395, GG450, GG495, OG550, RG610, RG665, and RG715 (Figure 1). This combination of long-pass cutoff filters produces DL curves for 8 wavelength ranges: all wavelengths, <395, 395–450, 450–495, 495–550, 550–610, 610–665, 665–715, and >715 nm, respectively. The wheel rotates counterclockwise.

2.2. Herbal Materials. All samples (37 raw root and Rhizome herbs) were collected by Jinan Jianlian Chinese medicinal herb store in Shandong province, China. An experienced herbalist (Professor Yuanbin Zhang) at the Shandong Academy of Medical Sciences identified the samples. The samples were divided into two groups based on their cold or heat properties (Table 1).

2.3. Preparation of Powder. The herbal samples were pulverized to 0.125–0.177 mm with a grinder (Jinsui Company, Zhejiang province, China, type JSP-350). Different diameters of herbal particles were selected with 125, 150, and 850 μm sieves (Yongkang Company, Zhejiang province, China). The sieved samples were subsequently placed in a 55 mm Petri dish and stored in a light-tight box (Chengsheng Company, Tianjin) with silica desiccant (Dingfeng Company, Zhejiang province, China, 3–5 mm Blue) for at least 16 hours prior to the DL measurements [20–23]. The water content of the samples was 6.4–7.9% according to the Chinese pharmacopoeia (2010). The room temperature was maintained at 20 ± 1 °C [24–26].

2.4. Data Analysis. Each sample was measured at least three times, and the decay kinetic data were averaged for subsequent data analysis. Statistical analyses were conducted using SPSS V.17 software (SPSS, USA). We used an independent-sample test to compare the DL kinetic parameters of the herbal samples. p values less than 0.05 were considered significant. For data fitting, Statistic 10 software was used.

3. Results

3.1. DL Reproducibility Testing. We selected two representative Chinese medicinal herbs with heat and cold properties to measure the reproducibility of the DL experiments. Each sample was analyzed with the powders of five independent batches of herbs under the same experimental conditions. Empty Petri dishes served as a control (Figure 2). Figure 2 also presents the long time decay emission of two Chinese medicinal herbs (Radix Sophorae Flavescentis (classified as cold) and Radix et Rhizome Ginseng Rubra (classified as heat)) following white light illumination. The reproducibility of the technique was good. The standard deviation of the DL intensity values was <5% in the first 5 seconds and 5–15% during subsequent time points. This difference occurred because the signal was lower at the later time points; thus, the influence of the noise increased.

3.2. Decay Probability Distribution of DL. The kinetics of the light-induced DL temporal trends have been described by a hyperbolic function law in multiple previous studies [27–30]:

\[ I(t) = \frac{I_0}{(1 + t/\tau)\beta}. \] (1)

Here, \( I_0 \) is the initial intensity following illumination, \( \beta \) is the index factor associated with the rate of decay, and \( \tau \) is the characteristic time, which is a constant specific to the sample.

Previous results implied that the DL of dried Chinese herbal materials is a complicated light emission process, which is similar to previous reports regarding sera and bacteria [31, 32]. To obtain comprehensive information regarding this complex decay process, we used a widely accepted approach in which time-resolved DL decays are described as continuous distributions of decay times or rate constants via the introduction of a probability density function \( f(y) \) as follows [33–35]:

\[ I(t) = \int_0^\infty A f(y) e^{-\gamma t} dy, \] (2)
Table 1: Nomenclature of selected cold and heat Chinese medicinal herbs.

| Pharmaceutical name | Chinese Pin Yin       | Latin botanical name               | Plant part used |
|----------------------|-----------------------|-----------------------------------|----------------|
| Radix Pulsatillae    | Bai Tou Weng          | Pulsatilla chinensis (Bge.) Regel.| Root           |
| Radix Dichroae       | Chang Shan            | Dichroa febrifuga Lour.           | Root           |
| Radix Stephaniae Tetrandrae | Fang Ji            | Stephania tetrandra S. Moore.     | Root           |
| Radix Scutellariae   | Huang Qin             | Scutellaria baicalensis Georgi.   | Root           |
| Radix Rhapontici     | Lou Lu                | Rhaponticum uniflorum (L.) DC.    | Root           |
| Radix Changii        | Ming Dang Shen        | Changium smyrnoides Wolf.         | Root           |
| Radix Curcumae       | Yu Jin                | Curcuma wenyujin Y. H. Chen et C. Ling. | Root       |
| Radix Arnebiae       | Zi Cao                | Lithospermum erthyrorhizzon Sien. et Zucc. | Root       |
| Radix Scrophulariae  | Xuan Shen             | Scrophularia ningpoensis Hemsl.   | Root           |
| Radix Paeoniae Alba  | Bai Shao              | Paeonia lactiflora Pall.          | Root           |
| Radix Trichosanthis  | Tian Hua Fen          | Trichosanthes kirilowii Maxim.    | Root           |
| Radix Puerariae      | Ge Gen                | Pueraria lobata (Willd.) Ohwi.    | Root           |
| Radix Panacis Quinquefolii | Xi Yang Shen      | Panax quinquefolia L.             | Root           |
| Radix Paeoniae Rubra | Chi Shao              | Paeonia lactiflora Pall.          | Root           |
| Radix Peucedani      | Qian Hu               | Peucedanum praeruptorum Dunn.     | Root           |
| Radix Stellariae     | Yin Chai Hu           | Stellaria dichotoma L. var. lanceolata Bge. | Root       |
| Radix Sophorae Flavescentis | Ku Shen            | Sophora flavescens Ait.           | Root           |
| Radix Ophiopogonis   | Mai Dong              | Ophiopogon japonicus (Thunb.) Ker-Gawl. | Root       |
| Radix Adenophorae    | Nan Sha Shen          | Adenophora tetraphylla (Thunb.) Fisch. | Root       |
| Rhizoma Phragmitis   | Lu Gen                | Phragmites communis (L.) Trin.    | Rhizome        |
| Rhizoma Anemarrhenae | Zhi Mu                | Anemarrhena asphodeloides Bge.    | Rhizome        |
| Rhizoma Belamcandae  | She Gan               | Belamcanda chinensis (L.) DC.     | Rhizome        |
| Radix et Rhizome Polygoni Cuspidati | Hu Zhang | Polygonum cuspidatum Sieb. et Zucc. | Rhizome and root |
| Radix Morindae Officinalis | Ba Ji Tian      | Morinda officinalis How.           | Root           |
| Radix Angelicae Dahuricae | Bai Zhi       | Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. F. | Root       |
| Radix Vladimiriae    | Chuan Mu Xiang        | Vladimira souliei (Franch.) Ling. | Root           |
| Radix Angelicae Sinensis | Dang Gui         | Angelica sinensis (Oliv.) Diels.  | Root           |
| Radix Linderae       | Wu Yao                | Lindera strychnifolia (Sieb. et Zucc.) Vill. | Root       |
| Radix Polygalae      | Yuan Zhi              | Polygala tenufolia Willd.         | Root           |
| Radix Stemonae       | Bai Bu                | Stemona sessilifolia (Miq.) Miq.  | Root           |
| Radix Angelicae Pubescentis | Du Huo            | Angelica pubescens Maxim. F. biserrata. | Root       |
| Radix Saposhnikoviae | Fang Feng             | Saposhnikovia divaricata (Turez.) Schischk. | Root       |
| Radix Astragali      | Hunag Qi              | Astragalus membranaceus (Fisch.) Bge. | Root       |
| Rhizoma Atractylodes Macrocephalae | Bai Zhu      | Atractylodes macrocephala Koidz.  | Rhizome        |
| Rhizome Galanga      | Gao Liang Jiang       | Alpinia officinarum Hance.        | Rhizome        |
| Radix et Rhizome Ginseng Rubra | Hong Shen     | Panax ginseng C. A. Mey.          | Rhizome and root |
| Radix et Rhizome Asteris | Zi Wan          | Aster tataricus L. f.             | Rhizome and root |
where $A$ is a normalizing constant and $\gamma$ is a rate constant of the decay process. Based on (1) and (2), we obtain

\[ \frac{I_0}{(1 + t/\tau)^\beta} = \int_0^\infty A f(y) e^{-\gamma t} dy. \]  

Therefore, the decay probability distribution $f(y)$ may be obtained through anti-Laplace transform processing:

\[ f(y) = \frac{\tau^\beta y^{\beta-1} e^{-\gamma \tau}}{\Gamma(\beta)}, \]  

where $\Gamma(\beta) = (\beta - 1)!$. Based on (4), decay probability distributions were created for 13 heat herbs and 24 cold herbs (Figure 3(a)). The cold and heat groups have substantially different decay probability distributions. The difference may also be correlated with the different ages and growth locations or postharvest processing. This leads to various complex reactions, including changes in the chemical makeup of the herbs and internal structural changes. Ultimately, external influences will be reflected in the overall efficacy of the TCM. To analyze the differences between the heat and cold Chinese
According to (1), three parameters, including $I_0$, $\beta$, and $\tau$, may be obtained by fitting the experimental data. According to (5), $P_{\text{max}}$ represents the peak value, and $\gamma_{\text{max}}$ represents the peak decay rate. As shown in Figures 3(b) and 3(c), the average of $P_{\text{max}}$ is $0.18 \pm 0.03$ of 23 cold herbs, which is substantially less than the average of $P_{\text{max}}$ of 14 heat herbs ($0.25 \pm 0.06$) and significant at $p = 0.044$. In addition, the average of $\gamma_{\text{max}}$ is $3.28 \pm 0.70$ from the cold herbs which was increased compared with the heat herbs ($2.54 \pm 0.25$) and significant at $p = 0.03$.

3.3. DL Emission Spectra of Chinese Medicinal Herbs.

Another intrinsic parameter that could be used to develop a strategy of discrimination is based on the measurement of emission spectra. Our initial findings are that the DL emission spectra of different properties of Chinese herbal materials are also with obvious difference. We used 7 different long-pass filters (as described in Section 2.1) to measure DL. Different spectra were collected based on the filter set used. Along with the filter wheel rotation, the emission photons can be obtained in different spectral range depending on the filter. In order to analyze the spectral components of emission spectrum, we calculated the photon radiation of different spectral range mentioned in Section 2.1. For example, by the photons of G450 minus G395, we can get the photons spectral range 395 nm–450 nm. We normalized the spectra to compare the peak locations by dividing the photon counts at each wavelength by the total number of photons. The peak location is characteristic of the herb. Figure 4 shows the average values and standard errors for the spectra of the heat and cold herbs. The most common characteristic spectral behavior is located from 550 to 610 nm. In comparison with the heat herbs, the cold herbs have a higher ratio range from 350 to 610 nm, and the heat herbs have a higher ratio range from 610 to 715 nm. These trends in the spectral distribution may be associated with the cold and heat properties of Chinese medicinal herbs.

4. Discussion

Many groups have used analytical techniques and clinical tools to investigate Chinese medicinal herbs. However, to date, modern TCM studies have not resulted in breakthroughs because of conceptual and methodological limitations. Here, we demonstrate how DL may represent an important tool to overcome some of these challenges.

The traditional analysis methods used in TCM are LC-MS and GC-MS. These approaches measure chemical compositions, including active ingredients. In general, the chemical composition governs the therapeutic properties of Chinese herbal materials. However, many earlier investigations have demonstrated that some Chinese medicinal herbs with similar chemical compositions exhibit different therapeutic properties [36, 37]. This finding may be because the entirety of the chemical repertoire in the herb is not assayed because of losses during sample preparation or low constituent abundance [38, 39]. This issue prevents a complete characterization of Chinese medicinal herbs. Moreover, traditional chemical methods emphasize a unique and specific chemical composition, which is in sharp contrast to the holistic view at the heart of TCM.
To overcome these limitations, we proposed the use of DL to characterize herbs because of its many advantages. First, it is fast, convenient, and affordable. Sample processing is limited to a simple grinding step. Samples may be immediately tested after grinding without additional chemical reagents. Moreover, very low detection limits are possible because of the sensitivity of PMTs. Importantly, this approach treats the herb as a whole, complex, and open system, which is how the body will metabolize them following consumption [40]. This approach is in sharp contrast to the reductionist approach taken in a chromatographic scheme.

5. Conclusion

We report a repeatable DL measurement protocol for dried Chinese medicinal herbs. We used this approach to analyze heat and cold Chinese medicinal herbs and identified a significant difference in the decay probability distribution between the two sample types. The peak decay rate ($\gamma_{\text{max}}$) and the peak weight value ($P_{\text{max}}$) offered explicit discrimination between the cold and heat property herbs. The spectral behavior trends were also different and may indicate an underlying mechanism of action in TCM. Nevertheless, these findings require further validation with additional samples.

In conclusion, DL is a novel tool used to investigate materials. DL offers comprehensive information regarding both chemical constituents and energy. It is a direct, rapid, and cumulative assay that provides novel information regarding the biological nature of herbal medicines.

Competing Interests

The authors declare that they have no competing interests.
Figure 4: Spectral distribution of DL emission of 37 peaks found in the herbs normalized to their sum.

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