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Development of personalized health monitoring using ultra-weak photon emission based on systems medicine concepts

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Chapter 6

Characterization of the therapeutic properties of Chinese herbal materials by measuring delayed luminescence and dendritic cell-based immunomodulatory response

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Abstract

Based on the traditional Chinese medicine theory, the Chinese pharmacopeia assigns a therapeutic description of "taste" to all herbs; thus, a herb's "taste" is valued in traditional Chinese medicine as a major ethnopharmacological category and reflects the herb's therapeutic properties. These properties guide the practitioner with respect to preparing a specific herbal formula in order to provide each patient with a personalized intervention. The key challenge in evidence-based medicine is to characterize herbal therapeutic properties from a multi-target, multi-dimensional systems pharmacology perspective. Here, we used delayed luminescence (DL, the slowly decaying emission of photons following excitation with light) as a rapid, direct, highly sensitive indicator to characterize the properties of herbal medicines. The DL parameters were able to reliably identify a specific category of herbal materials with the so-called "sweet" taste. To support the DL results and provide biological relevance to the DL results, we used a murine bone marrow-derived dendritic cell–based assay to examine the immunomodulatory effects of herbal extracts from various "taste" categories. Our results indicate that DL may serve as a robust and sensitive tool for evaluating the therapeutic properties of herbs based on the traditional Chinese medicine classification of "taste". Thus, DL provides a promising technological platform for investigating the properties of Chinese herbal medicines both qualitatively and quantitatively.

Key words: Chinese herbal medicine, therapeutic classification, delayed luminescence, immunity, dendritic cell

1. Introduction

Chinese herbal medicine has been used in China for thousands of years to maintain health and treat disease.[1] In this long history, practitioners of traditional Chinese medicine have accumulated a wealth of knowledge regarding herbal therapeutic effects based on clinical observations, resulting in the classification of herbal medicines into specific therapeutic categories.[1,2] Chinese herbal medicines are traditionally classified according to the sensations they evoke and the patient's response, resulting in descriptive characterizations such as taste, warm/cold, and toxic/non-toxic.[1–3] The taste category includes descriptors based on the perception in the mouth and include sweet, bitter, pungent, salty, and sour.[4] Our current understanding of the herbal descriptor "taste" developed from a long history of clinical experience and is linked to specific therapeutic properties in humans.[3,4] Hence, in traditional Chinese medicine, herbs are not necessarily classified according to their perception in the mouth, but rather according to their therapeutic properties in the human body.[4] Thus, the five taste descriptors have been used to classify the specific therapeutic properties and pharmacological actions of Chinese herbal medicines in clinical practice.[5] As a result, the taste descriptors of some herbal medicines currently described in the herbal materia medica can differ from how they actually taste in the mouth.[6]

Based on the classification of taste in traditional Chinese medicine, herbs with the same taste descriptor generally possess similar therapeutic properties, and herbs with different taste descriptors generally have different therapeutic properties.[6] For example, the so-called "sweet" class of herbs is associated with a tonic effect that can nourish the body, hence promoting a healthy status, boosting the immune system, and helping fight the aging process.[4,7,8] On the other hand, herbs in the so-called "pungent" class eliminate pathological agents and therefore treat the corresponding symptoms (e.g., stagnation) by promoting the circulation of energy and blood.[4]

Herbs in the so-called "bitter" class have heat-cleansing and detoxification effects and are used to treat constipation, inflammation, infection, and other conditions.[5,9]

Herbs that have a single taste descriptor generally have basic therapeutic properties. However, many herbs belong to two or more taste classes and therefore have a wider therapeutic range than herbs with a single taste descriptor.[5] These taste descriptor–based therapeutic classifications are often used to help the practitioner prepare specific herbal formulas in order to achieve personalized intervention.[1] Therefore, the taste descriptor is an important concept for understanding an herb's therapeutic effect and clinical application, and it is listed both in Chinese medicine textbooks and in the Chinese Pharmacopoeia.[10,11] Interestingly, traditional Indian medicine (i.e., Ayurveda) also uses taste descriptors to indicate the pharmacological activity of herbal medicine.[12,13] Thus, both Ayurveda and traditional Chinese medicine use a common system of taste descriptors. In addition, statistical analyses support the use of herbal taste descriptors for predicting the pharmacological activity of herbs.[12] However, evidence-based scientific data is still needed in order to understand the therapeutic properties of herbal medicines based on taste descriptors.

Delayed luminescence (DL) is the long-term decay of weak photon emissions from materials following exposure to light with a wavelength of 400–800 nm.[14] DL provides a holistic, integrated, comprehensive method for measuring materials and biological systems, and provides a direct, rapid, and sensitive indicator of a wide range of processes, including food quality, seed germination, and cancerous cells.[15–17] Recently, we used DL to study the features of dry powders prepared from Chinese herbal materials.[14,18] The results suggest that specific DL properties can be used to indicate differences in herbal materials prepared under different conditions, including the processing method and the age of the herb.[18] Importantly, DL can be used to detect differences in the overall signatures of a given herb grown under various environmental conditions, and distinct DL properties have been correlated to the specific bioactive constituents extracted from these herbal samples.[14] These

differences in DL properties indicate the presence of different bioactive constituents as a result of environmental factors.[14] Because these studies confirm that DL can reflect herbal characteristics at the systems level,[14] we hypothesized that DL may be used to increase our understanding of herbal therapeutic properties based on the taste descriptor.

Here, we measured DL in Chinese herbal materials with different taste descriptors. In addition, to support our finding of distinct clusters of sweet descriptors and other herbal taste descriptors based on our DL data, we used murine bone marrow-derived dendritic cells (DCs) in an assay to examine the immunomodulatory effect of herbal extracts from various taste descriptor categories. DCs are specialized leukocytes that play a key role in initiating the adaptive immune response,[19] and the production of cytokines such as TNFα and IL-6 by DCs has been used as an indicator of the immunomodulatory capacity of herbal medicines.[20] The results obtained with our DC-based immunomodulatory assay generally support the results of our DL experiments; therefore, DL may provide both qualitative and quantitative insights into the therapeutic properties of herbal medicines.

2. Materials and Methods

2.1 Herbal materials

A total of 90 herbal materials (roots and/or rhizomes), ginseng leaves, and ginseng flowers were purchased in five batches from TongRenTang Co., Ltd. (Beijing, China). These 90 herbal medicines are listed in Table 1 and are classified according to six taste descriptor groups—sweet, bitter, pungent, sweet & bitter, sweet & pungent, and bitter & pungent—in accordance with the 2015 Chinese Pharmacopoeia.[11] The identities of all herbal samples were verified by Dr. Wen-Te Chang (China Medical University) and were deposited at China Medical University (Taichung, Taiwan).

Table 1. List of the 90 herbal medicines used in this study, including the Latin names and taste classifications.

ID	Name (English)	Name (Latin)	Taste descriptor
S1*	ASTRAGALI RADIX	*Astragulus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	Sweet
S2	HEDYSARI RADIX	*Hedysarum polybotrys* Hand. - Mazz	Sweet
S3	GLYCYRRHIZAE RADIX ET RHIZOMA	*Glycyrrhiza uralensis* Fisch.	Sweet
S4*	CODONOPSIS RADIX	*Codonopsis pilosula* (Franch.) Nannf.	Sweet
S5	POLYGONATI RHIZOMA	*Polygonatum kingianum* Coll. et Hemsl	Sweet
S6	DIOSCOREAE RHIZOMA	*Dioscorea opposita* Thunb.	Sweet
S7	GASTRODIAE RHIZOMA	*Gastrodia elata* Bl.	Sweet
S8	ADENOPHORAE RADIX	*Adenophora stricta* Miq.	Sweet
S9	STELLARIAE RADIX	*Stellaria dichotoma* L. var. *lanceolata* Bge.	Sweet
S10*	POLYGONATI ODORATI RHIZOMA	*Polygonatum odoratum* (Mill.) Druce	Sweet
S11	IMPERATAE RHIZOMA	*Imperata cylindrica* Beauv. var. *major* (Nees) C. E. Hubb.	Sweet
S12	REHMANNIAE RADIX	*Rehmannia glutinosa* Libosch.	Sweet
S13	PHRAGMITIS RHIZOMA	*Phragmites communis* Trin.	Sweet
S14	NOTOGINSENG RADIX ET RHIZOMA	*Panax notoginseng* (Burk.) F.H. Chen	Sweet
S15*	GINSENG RADIX ET RHIZOMA RUBRA	*Panax ginseng* C. A. Mey.	Sweet
S16	GINSENG RADIX ET RHIZOMA	*Panax ginseng* C. A. Mey.	Sweet
S17*	PSEUDOSTELLARIAE RADIX	*Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm.	Sweet
S18	CYATHULAE RADIX	*Cyathula officinalis* Kuan	Sweet
S19	PANACIS QUINQUEFOLII RADIX	*Panax quinquefolium* L.	Sweet
S20	CHANGII RADIX	*Changium smyrnioides* Wolff	Sweet
S21*	OPHIOPOGONIS RADIX	*Ophiopogon japonicas* (L.f) Ker-Gawl.	Sweet
S22	TRICHOSANTHIS RADIX	*Trichosanthes kirilowii* Maxim.	Sweet
S23	GLEHNIAE RADIX	*Glehnia littoralis* Fr. Schmidtex Miq.	Sweet
P1	CURCULIGINIS RHIZOMA	*Curculigo orchioides* Gaertn.	Pungent
P2*	ANEMONES RADDEANAE RHIZOMA	*Anemone raddeana* Regel	Pungent
P3*	ALPINIAE OFFICINARUM RHIZOMA	*Alpinia officinarum* Hance	Pungent
P4	ZINGIBERIS RHIZOMA	*Zingiber officinale* Rosc.	Pungent
P5	ANGELICAE DAHURICAE RADIX	*Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f.	Pungent
P6*	CHUANXIONG RHIZOMA	*Ligusticum chuanxiong* Hort.	Pungent
P7	ASARI RADIX ET RHIZOMA	*Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag.	Pungent
P8	CYNANCHI PANICULATI RADIX ET RHIZOMA	*Cynanchum paniculatum* (Bge.) Kitag.	Pungent
P9*	LIGUSTICI RHIZOMA ET RADIX	*Ligusticum sinense* Oliv.	Pungent
P10	KAEMPFERIAE RHIZOMA	*Kaempferia galanga* L.	Pungent
P11*	LINDERAE RADIX	*Lindera aggregata* (Sims) Kosterm.	Pungent
P12	CYPERI RHIZOMA	*Cyperus rotundus* L.	Pungent
P13	CIMICIFUGAE RHIZOMA	*Cimicifuga foetida* L.	Pungent
B1	DRYNARIAE RHIZOMA	*Drynaria fortunei* (Kunze) J. Sm.	Bitter
B2	DIOSCOREAE SPONGIOSAE RHIZOMA	*Dioscorea spongiosa* J.Q.Xi,M.Mizuno et W.L.Zhao	Bitter
B3*	SALVIAE MILTIORRHIZAE RADIX ET RHIZOMA	*Salvia Miltiorrhiza* Bge.	Bitter
B4	PAEONIAE RADIX RUBRA	*Paeonia lactiflora* pall.	Bitter
B5	PARIDIS RHIZOMA	*Paris polyphylla* Smith var.*yunnanensis* (Franch.) Hand. -Mazz	Bitter
B6	AMPELOPSIS RADIX	*Ampelopsis japonica* (Thunb.) Makino	Bitter
B7	POLYGONI CUSPIDATI RHIZOMA ET RADIX	*Polygonum cuspidatum* Sieb. et Zucc.	Bitter
B8*	RHEI RADIX ET RHIZOMA	*Rheum palmatum* L.	Bitter
B9	PULSATILLAE RADIX	*Pulsatilla chinensis (Bge.) Regel*	Bitter

(continued on next page)

Table 1. *(continued)*

ID	Name (English)	Name (Latin)	Taste descriptor
B10	KNOXIAE RADIX	*Knoxia valerianoides* Thorel et Pitard	Bitter
B11	SOPHORAE FLAVESCENTIS RADIX	*Sophora flavescens* Ait.	Bitter
B12	ISATIDIS RADIX	*Isatis indigotica* Fort.	Bitter
B13*	PICRORHIZAE RHIZOMA	*Picrorhiza scrophulariiflora* Pennell	Bitter
B14	RUBIAE RADIX ET RHIZOMA	*Rubia cordifolia* L.	Bitter
B15	BELAMCANDAE RHIZOMA	*Belamcanda chinensis* (L.) DC.	Bitter
B16*	SCUTELLARIAE RADIX	*Scutellaria baicalensis* Georgi	Bitter
B17*	COPTIDIS RHIZOMA	*Coptis chinensis* Franch.	Bitter
B18	PHYTOLACCAE RADIX	*Phytolacca acinosa* Roxb.	Bitter
B19	STEPHANIAE TETRANDRAE RADIX	*Stephania Tetrandra* S. Moore	Bitter
B20	PHAPONTICI RADIX	*Rhaponticum uniflorum (L.) DC.*	Bitter
BP1	ACONITI RADIX	*Aconitum carmichaelii* Debx.	Bitter & Pungent
BP2	ACONITI KUSNEZOFFII RADIX COCTA	*Aconitum kusnezoffii* Reichb.	Bitter & Pungent
BP3	AUCKLANDIAE RADIX	*Aucklandia lappa* Decne.	Bitter & Pungent
BP4	NOTOPTERYGII RHIZOMA ET RADIX	*Notopterygium incisum* Ting ex H.T. Chang	Bitter & Pungent
BP5	CURCUMAE LONGAE RHIZOMA	*Curcuma Longa* L.	Bitter & Pungent
BP6	ASTERIS RADIX ET RHIZOMA	*Aster tataricus* L.f.	Bitter & Pungent
BP7	ATRACTYLODIS RHIZOMA	*Atractylodes lancea* (Thunb.) DC.	Bitter & Pungent
BP8	ACORI TATARINOWII RHIZOMA	*Acorus tatarinowii* Schott	Bitter & Pungent
BP9	CYNANCHI STAUNTONII RHIZOMA ET RADIX	*Cynanchum stauntonii* (Decne.) Schltr.ex Lévl.	Bitter & Pungent
BP10	ANGELICAE PUBESCENTIS RADIX	*Angelica Pubescens* Maxim.f.*biserrata* Shan et Yuan	Bitter & Pungent
BP11	GENTIANAE MACROPHYLLAE RADIX	*Gentiana macrophylla* Pall.	Bitter & Pungent
BP12	BUPLEURI RADIX	*Bupleurum chinense* DC.	Bitter & Pungent
BP13	CURCUMAE RADIX	*Curcuma wenyujin* Y. H. Chen et C. Ling	Bitter & Pungent
BP14	HOMALOMENAE RHIZOMA	*Homalomena occulta* (Lour.) Schott	Bitter & Pungent
BP15	POLYGALAE RADIX	*Polygala tenuifolia* Willd.	Bitter & Pungent
BP16	DIPSACI RADIX	*Dipsacus asper* Wall. ex Henry	Bitter & Pungent
BP17	PLATYCODONIS RADIX	*Platycodon grandiflorum* (Jacq.) A.DC.	Bitter & Pungent
BP18	ZANTHOXYLI RADIX	*Zanthoxylum nitidum* (Roxb.) DC.	Bitter & Pungent
BP19	PEUCEDANI RADIX	*Peucedanum praeruptorum* Dunn	Bitter & Pungent
BP20	DICHROAE RADIX	*Dichroa febrifuga* Lour.	Bitter & Pungent
SP1	ACONITI LATERALIS RADIX PRAEPARAIA	*Aconitum carmichaelii* Debx.	Sweet & Pungent
SP2	NARDOSTACHYOS RADIX ET RHIZOMA	*Nardostachys jatamansi* DC.	Sweet & Pungent
SP3	SAPOSHNIKOVIAE RADIX	*Saposhnikovia divaricata* (Turcz.) Schischk.	Sweet & Pungent
SP4	ANGELICAE SINENSIS RADIX	*Angelica sinensis* (Oliv.) Diels	Sweet & Pungent
SP5	RANUNCULI TERNATI RADIX	*Ranunculus ternatus* Thunb.	Sweet & Pungent
SP6	MORINDAE OFFICINALIS RADIX	*Morinda officinalis* How	Sweet & Pungent
SP7	PUERARIAE THOMSONII RADIX	*Pueraria thomsonii* Benth.	Sweet & Pungent
SP8	PUERARIAE LOBATAE RADIX	*Pueraria lobata* (Willd.) Ohwi	Sweet & Pungent
SB1	STEMONAE RADIX	*Stemona sessilifolia* (Miq.) Miq.	Sweet & Bitter
SB2	ASPARAGI RADIX	*Asparagus cochinchinensis* (Lour.) Merr.	Sweet & Bitter
SB3	SEMIAQUILEGIAE RADIX	*Semiaquilegia adoxoides* (DC.) Makino	Sweet & Bitter
SB4	CIBOTII RHIZOMA	*Cibotium barometz* (L.) J. Sm.	Sweet & Bitter
SB5	ATRACTYLODIS MACROCEPHALAE RHIZOMA	*Atractylodes macrocephala* Koidz.	Sweet & Bitter
SB6	ANEMARRHENAE RHIZOMA	*Anemarrhena asphodeloides* Bge.	Sweet & Bitter

*, Herbs that were examined in the in vitro cell assay. Taste descriptors are based on the 2015 Chinese Pharmacopoeia.[11]

2.2. Delayed luminescence (DL)

2.2.1. Sample preparation

Each herbal sample was crushed using a model QE-100 grinder (Yili Company, Zhejiang Province, China) and passed through a standard sieve to obtain150-μm particles.[18] These herbal samples were kept in a dark, light-tight box containing 35-mm silica gel (Boom BV, Meppel, the Netherlands) at room temperature for 16 h before DL measurements were conducted.[18]

2.2.2. DL measurement

DL was measured in the herbal samples as described previously.[18] The instrument used to measure DL was obtained from Meluna Research (Geldermalsen, the Netherlands) and included a photomultiplier tube (PMT) (type 9558QB; Electron Tubes Enterprises Ltd., Ruislip, UK) vertically positioned on a dark sample chamber kept at 22°C. The PMT contains a cathode end (51 mm diameter) with sensitivity at 160–870 nm. The PMT was cooled to −25°C in order to reduce the dark count rate to 10 counts per second. The DL signal was amplified using a type 9301 fast preamplifier (ORTEC, Oak Ridge, TN). Data were acquired using a personal computer containing a model 6602 counting card (National Instruments, Austin, TX). Each herbal sample (1 g) was placed in a plastic Petri dish (35-mm diameter) and excited for 10 s using a model 284-2812 white halogen light source (Philips, Germany). For each sample batch, the DL signal was measured three consecutive times, and a total of fifteen measurements in five batches were used to examine the DL properties of that particular herbal medicine. The DL decay signature was obtained by recording the number of photon counts in consecutive 0.05-s periods for a total of 60 s, yielding a total of 1200 data points.

2.3 Dendritic cell assay

2.3.1 Preparation of extracts

Sixteen herbal samples were tested using the DC assay (Table 1). Each herbal sample was divided into three batches, and each batch of a specific herb was used to prepare four 4-g samples; hence, each herbal sample was divided into 12 independent samples, which were crushed into powder form using a type QE-100 grinder (Yili Company, Zhejiang Province, China). Each powdered herbal sample was extracted with 80 ml distilled water at 100°C for 1 hour. The resulting extraction was then centrifuged, filtered through No. 1 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and concentrated using a model R-210 rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland).[20] Thereafter, the water extract was mixed with 95% ethanol at a ratio of 1:5.7 (v:v), chilled at 4°C overnight, and then centrifuged at 5000 rpm for 30 mins to precipitate polysaccharides and proteins. The resulting supernatant was then filtered through No. 1 filter paper (Toyo Roshi Kaisha, Ltd.). Finally, 12 independent water extracts were obtained from each original herbal sample, dried in a vacuum evaporator overnight, and stored at 4°C until further use in the DC assay.

2.3.2 Mice

ICR mice (8-12 weeks of age) were purchased from BioLASCO Taiwan Co., Ltd (Taipei, Taiwan). All animals were housed in a specific pathogen-free facility at the Animal Center of China Medical University (Taichung, Taiwan) and handled in accordance with the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan).[20]

2.3.3 Preparation of mouse dendritic cells (DCs)

The methods for preparing and culturing bone marrow-derived dendritic cells have been described previously.[20] In brief, bone marrow cells were isolated from the tibias and femurs. After the red blood cells were removed, the remaining cells were seeded on 6-well culture plates (Costar) with RPMI 1640 medium (Hyclone) supplemented with HEPES, penicillin/streptomycin (Gibco), 10% fetal bovine serum (Hyclone),

recombinant mouse GM-CSF (10 ng/ml, PeproTech), and IL-4 (10 ng/ml, PeproTech). After 7 days in culture, the DCs were collected and used for the experiments described below.

2.3.4 Effects of herbal extracts on DCs

Each herbal extract was dissolved in DMSO to prepare a 500 mg/ml solution. DCs were treated with each herbal extract at 500 μg/ml in the absence or presence of 100 ng/ml lipopolysaccharide (LPS) for 6 h. The concentrations of TNFα and IL-6 in the medium were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA) in accordance with the manufacturer's instructions.[21] Control standards (10 μg/ml in PBS) were used to treat DCs in the absence or presence of LPS.[20] Each herbal extract sample was tested in two independent experiments; thus, a total of 24 datasets for TNFα and IL-6 were obtained for each specific herb.

2.4 Data processing and statistical analysis

2.4.1 DL Properties

The DL decay curve for each sample, measured over a 60-second period, was fit to the following double-exponential function:[18]

$$y = y0 + A1e^{-\frac{x}{t1}} + A2e^{-\frac{x}{t2}}$$

where y0 is the final value of DL emission in the decay curve, A1 and A2 are the amplitudes of the exponential decay components, and t1 and t2 are time constants for the exponential decays.[18] The R package nnet (version 3.2.2) was used to perform the DL curve-fitting.[22] The median of each DL property from the fifteen measurements was calculated and used to represent the DL signature of each herbal medicine. One-way analysis of variance (ANOVA) with least significant difference (LSD) post hoc analysis[23] was used to compare the DL properties between the six

taste groups; differences were considered significant at $p<0.05$. Principal component analysis (PCA) was used to indicate the level of discrimination between DL properties, using the tools provided in the MetaboAnalyst software package (http://www.metaboanalyst.ca).[14,23]

2.4.2 Cytokines measured in the DC assay

To quantify the immunomodulatory effect of the herbal samples on DCs, the concentrations of the cytokines TNFα and IL-6 were normalized to the control standard (to measure activation) or the control standard plus LPS (to measure inhibition); these control samples were set to 100%. The percentage of each herbal sample relative to the control value was used to reflect the relative secretion of cytokines. Thereafter, a one-sample Student's *t*-test was used to analyze the difference between each specific herbal sample (or taste group) and control standard (or control standard plus LPS) using SPSS (version 23.0; IBM, Armonk, NY). Differences were considered significant at $p<0.05$.

3. Results

In traditional Chinese medicine, various components in plants can be used as herbal materials, including the roots and/or rhizomes, leaves, flowers, fruits, and/or seeds.[11] From a biological perspective, different parts of a plant play distinct roles in the plant's physiology.[24] Because DL can be used to detect the holistic signatures of plant materials,[25,26] we first investigated whether different parts of a plant have different DL signatures. Therefore, we measured the dry roots and rhizomes (in one sample), as well as the leaves and flowers of ginseng; the DL decay curves for the various dry materials are shown in Fig. 1. The roots and rhizomes sample produced a significantly different DL signature compared to the leaves and flowers; in particular, the DL features in the tail of the curve differ considerably. Therefore, in order to minimize differences in the DL signatures obtained from different parts of plant, we focused our analysis on the roots and/or rhizomes, as these two structures

grow beneath the soil, and the corresponding herbs are commonly used in traditional Chinese medicine. Table 1 lists the 90 Chinese herbal materials that were analyzed, their taste groups, and their main descriptors (sweet, bitter, or pungent), as well as the combinations of these three tastes in accordance with the 2015 Chinese Pharmacopoeia.[11]

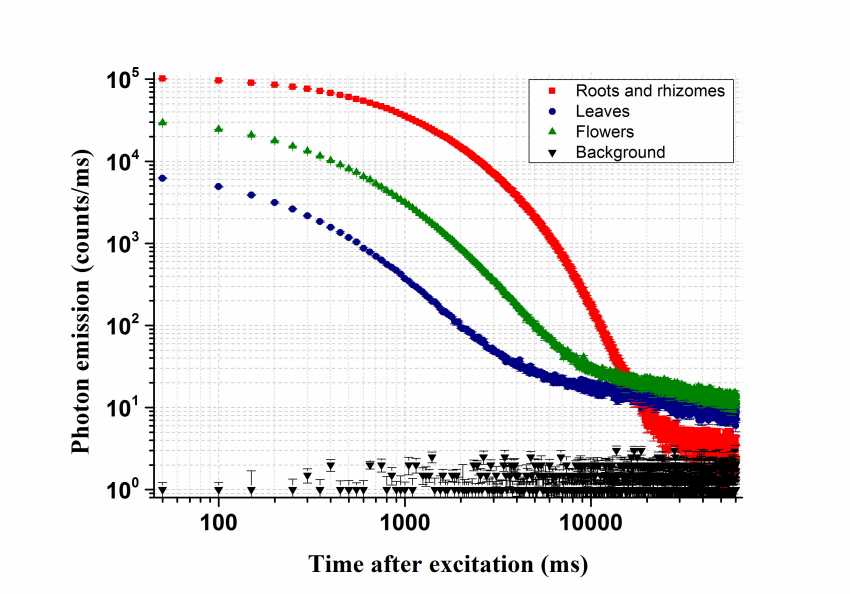

Fig. 1 DL decay curves for ginseng roots and rhizomes, ginseng leaves, and ginseng flowers. Data are plotted as the mean ± SEM. Note that the data are plotted on a log-log scale.

Next, we measured the DL profiles of these 90 herbal samples. The results show that herbal samples in the same taste descriptor category produce generally similar DL curves, with strikingly different curves between “sweet” herbs and “bitter” herbs (Fig. 2). To compare the overall DL curves obtained from the three individual taste descriptor groups, we pooled the data from the herbal samples within a specific taste descriptor group; these results are shown in Fig. 3. These results show that the DL dynamics in the “sweet” descriptor group differ from both the “pungent” and “bitter” taste groups; moreover, the “pungent” and “bitter” groups have similar DL decay profiles (Fig. 3).

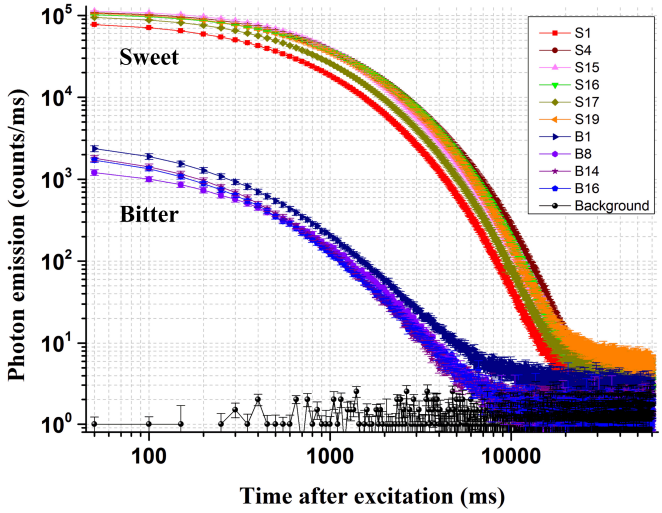

Fig. 2 DL decay curves of several herbal samples with "sweet" (S) descriptor and "bitter" (B) descriptor. Data are plotted as the mean ± SEM. Note that the data are plotted on a log-log scale. Each specific herbal sample is indicated by ID number (see Table 1).

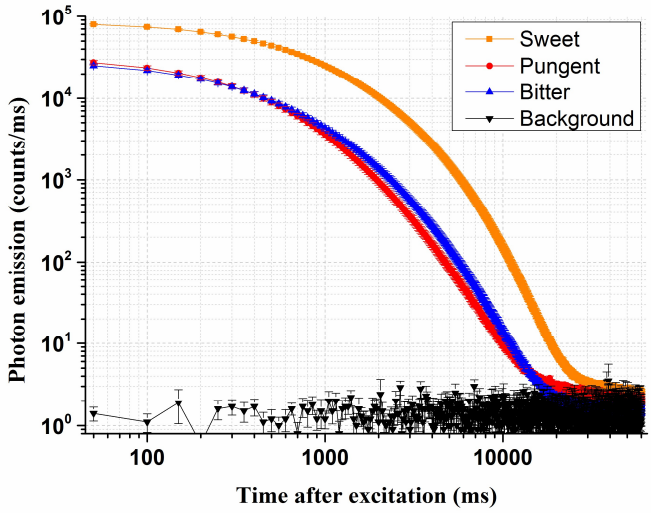

Fig. 3 DL decay curves for pooled samples from the sweet, bitter, and pungent groups. Data are plotted as the mean ± SEM. Note that the data are plotted on a log-log scale.

Five parameters were then derived from the DL curves by fitting the decay curves with a double-exponential function. We then compared these five parameters between all six taste groups using a one-way ANOVA; the results are summarized in Fig. 4. Fig. 4 reveals that the five parameters (i.e., A1, A2, t1, t2 and y0) can be used to differentiate between the "sweet" descriptor group and the other five groups.

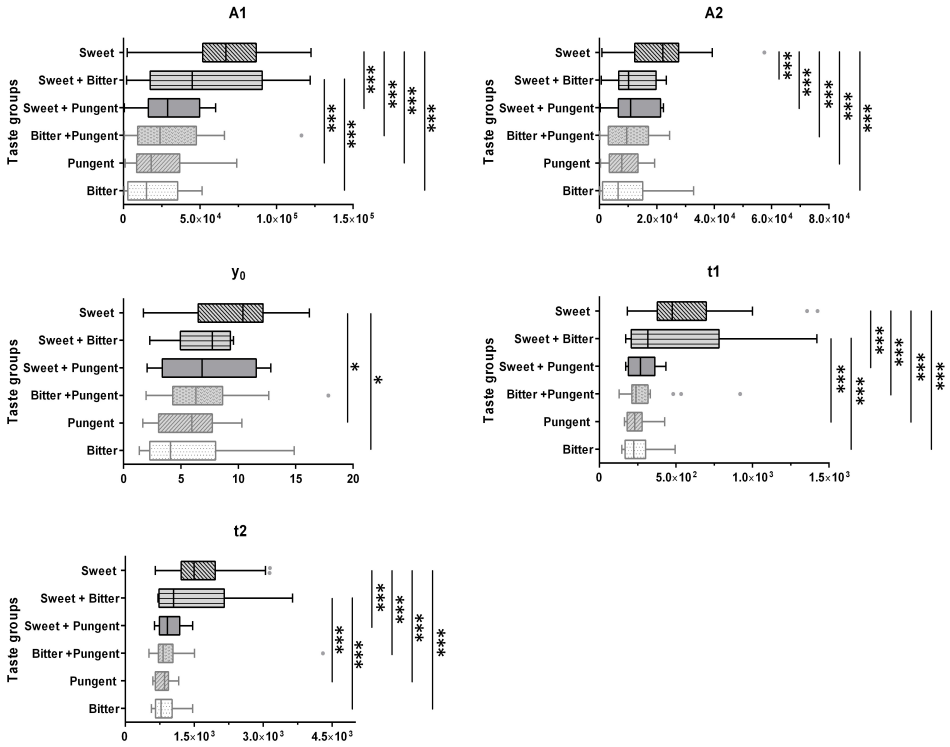

Fig. 4 Box plot summarizing the five DL properties measured in the six "taste" groups. *, $p<0.05$; ***, $p<0.001$ (one-way ANOVA with LSD).

To visualize the overall differences in DL properties between the various groups using an unsupervised method, we applied principal component analysis (PCA) for each herbal sample in the taste groups "sweet" and "bitter" (Fig. 5A) and in the taste groups "sweet" and "pungent" (Fig. 5B). Fig. 5A shows the PCA score plot using DL parameters in which PC1 and PC2 account for 70.9% and 20.4% of the total variance, respectively. This plot shows that the DL properties of the sweet descriptor

group and the bitter descriptor group generally form distinct clusters, with minor overlap. The DL parameters of five “sweet” herbal samples (S5, S6, S12, S14, and S18) were misclassified as belonging to the “bitter” descriptor group, whereas two “bitter” herbal samples (B4 and B12) were misclassified as belonging to the “sweet” descriptor group. Fig. 5B shows the PCA score plot in which PC1 and PC2 account for 67% and 23.6% of the total variance, respectively. These results show that the DL properties of the sweet descriptor group and the pungent descriptor group generally form two distinct clusters, with some overlap. None of the “pungent” herbal samples were misclassified as belonging to the “sweet” descriptor group, whereas five “sweet” herbal samples (S5, S6, S12, S14, and S18—the same five samples that were misclassified as belonging to the “bitter” group) were misclassified as belonging to the “pungent” descriptor group. No indication of separate PCA clusters was found between the “bitter” and “pungent” descriptor groups. Moreover, a PCA analysis between the “sweet” group and the “bitter & pungent” group revealed significant clusters between these two groups (Supplemental Fig. S1). We did not perform PCA analyses using the “sweet & pungent” descriptor group or the “sweet & bitter” descriptor group due to the relatively small numbers of herbal samples in these two groups. These results show that the “sweet” descriptor is unique among the descriptor groups with respect to both the DL curves and the DL parameters. Therefore, we conclude that DL can be used to differentiate between various taste descriptor classifications and can be used identify therapeutic properties in specific herbal materials at the systems level.

Next, to provide biological relevance to these findings, we performed an *in vitro* assay using mouse dendritic cells (DCs) and examined the immunomodulatory effects of select herbal samples. To reduce the complexity of herbal properties, we excluded the herbal materials with double taste descriptors (e.g., “sweet & bitter”, sweet & pungent”, etc.), and we examined 16 herbal materials with single taste descriptors.

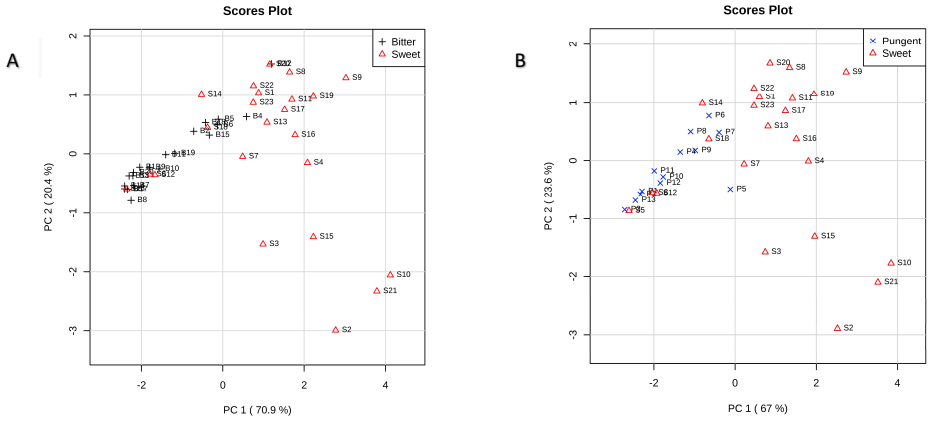

Fig. 5 PCA scores of the DL properties measured for the indicated "taste" herbs. A) "Sweet" herbs and "bitter" herbs are plotted. B) "Sweet" herbs and "pungent" herbs are plotted. Each symbol represents an individual herbal material with the corresponding ID number (see Table 1).

Water extraction is the traditional method used to prepare medicinal herbs and is well-suited to evaluating pharmacological effects based on clinic experience. In this study, we treated DCs using herbal water extract either in the absence or presence of LPS. These measurements were performed using two independent cell batches, and 12 measurements were obtained for each herbal sample in a specific cell batch.

To study the immunomodulatory response to a specific "taste" group as a whole, we pooled data from the individual herbal materials in each group. Fig. 6 summarizes the immunomodulatory responses of the three "taste" groups measured using secreted TNFα and IL-6. To study the ability of each group to inhibit the immune response, we first treated the cells with LPS, a potent activator of DCs.[19] Fig. 6A shows that both the "bitter" and "pungent" taste groups significantly inhibited the LPS-induced increase in TNFα and IL-6 secretion, with the pungent group having the strongest effect. In contrast, the "sweet" taste group caused higher IL-6 secretion compared to LPS alone (Fig. 6A). With respect to activation, Fig. 6B shows that both the "sweet" and "bitter" groups significantly increased TNFα secretion, whereas only the "sweet" group significantly increased IL-6 secretion. The "pungent" group

had no significant effect on either TNFα or IL-6 (Fig. 6B). In summary, these results show that herbs in the "sweet" group are primarily immunostimulatory, whereas herbs in the "bitter" and "pungent" groups are primarily immunosuppressive. These results generally correspond to the functional indications of herbal "taste" classifications based on traditional Chinese herbal medicine.[4]

Lastly, we examined the immunostimulatory and immunosuppressive effects of individual sweet, bitter, and pungent herbal medicines. With respect to the sweet herbal materials (Fig. 7A1), five samples—Codonopsis radix (S4), Polygonati odorati rhizoma (S10), Ginseng radix et rhizoma rubra (S15), Pseudostellariae radix (S17), and Ophiopogonis radix (S21) —significantly increased TNFα production, and three samples—Codonopsis radix (S4), Polygonati odorati rhizome (S10), and Ginseng radix et rhizoma rubra (S15)—significantly increased IL-6 production.

Moreover, two sweet herbs—Codonopsis radix (S4) and Ginseng radix et rhizoma rubra (S15)—significantly inhibited the effect of LPS on TNFα (Fig. 7A2). This result suggests that Codonopsis radix (S4) and Ginseng radix et rhizoma rubra (S15) can act as both an inhibitor and activator of the immune response. Interestingly, Astragali radix (S1) increased the secretion of TNFα in the presence of LPS but significantly decreased the secretion of IL-6 (Fig 7A2). In addition, Codonopsis radix (S4), Pseudostellariae radix (S17), and Ophiopogonis radix (S21) significantly inhibited TNFα in the presence of LPS, but significantly increased IL-6 secretion.

With respect to the "bitter" herbal samples, the majority did not significantly increase TNFα or IL-6 secretion (Fig. 7B1); indeed, two "bitter" herbs—Picrorhizae rhizoma (B13) and Coptidis rhizoma (B17)—led to significant decreases in TNFα and IL-6, respectively. Only one herb in the bitter group—Rhei radix et rhizoma (B8)—significantly increased TNFα secretion and significantly decreased IL-6 secretion (Fig. 7B1). A similar pattern was observed for this bitter herb with respect to inhibition in the presence of LPS—Rhei radix et rhizoma (B8) significantly increased TNFα secretion and significantly decreased IL-6 secretion (Fig. 7B2).

Moreover, the majority of bitter herbal samples significantly inhibited both TNFα and IL-6 (Fig. 7B2); Scutellariae (B16) and Coptidis (B17) had the strongest immunosuppressive effect among the five bitter herbal materials tested. Finally, the "pungent" herbal samples generally inhibited both TNFα and IL-6 secretion under both control (Fig. 7C1) and LPS (Fig. 7C2) conditions.

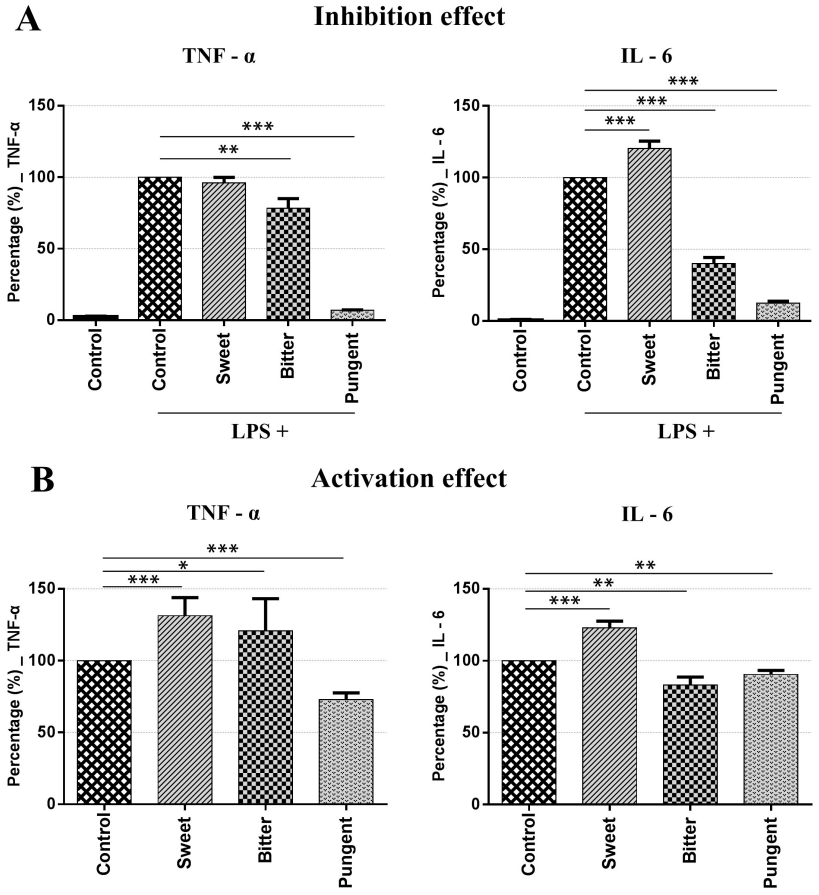

Fig. 6. Immunomodulatory effects of the various taste groups. A) Summary of the inhibitory effect of each taste group compared to cells stimulated with LPS. B) Summary of the stimulatory effect of each taste group compared to control (unstimulated) cells. The secretion of TNFα and IL-6 was measure using ELISA and is plotted relative to the respective control. Data are plotted as the mean ± SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (one-sample Student's t-test).

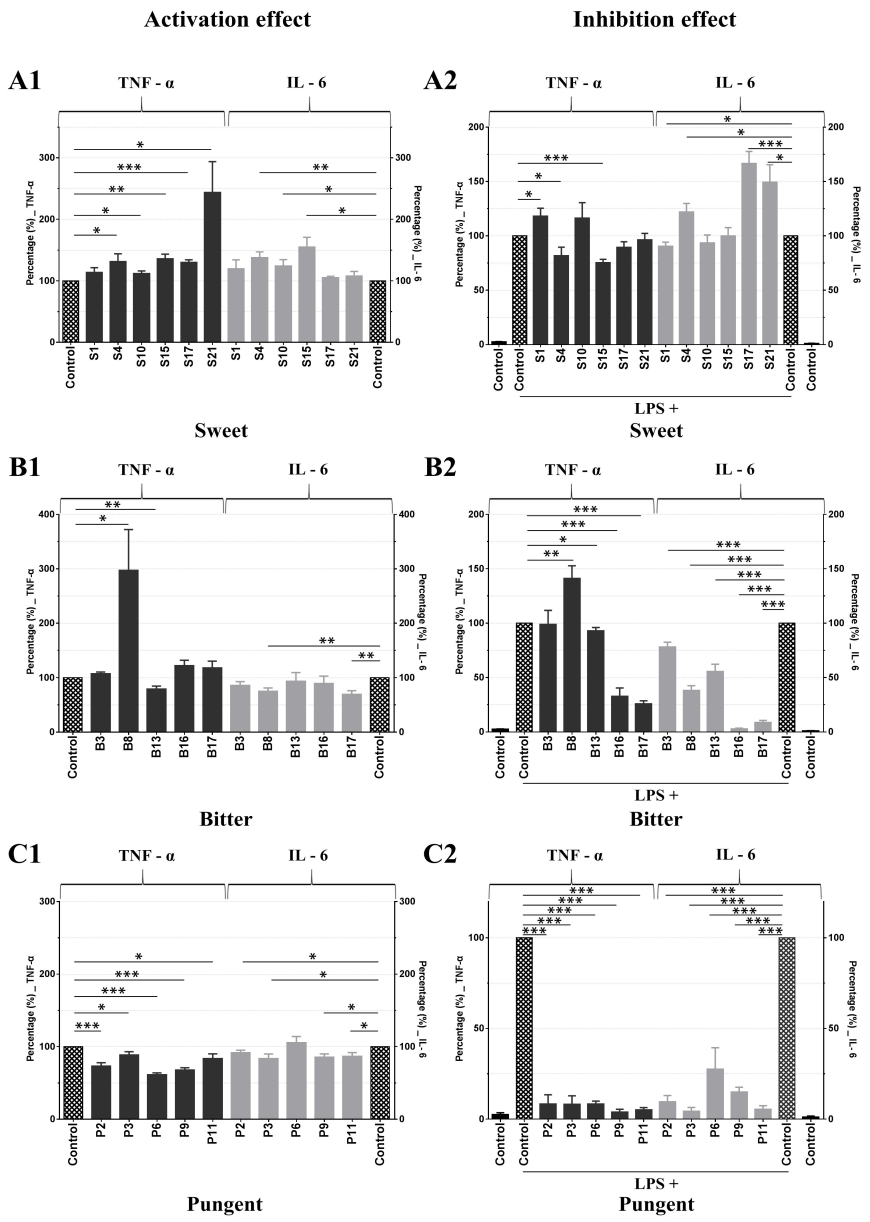

Fig. 7. Immunomodulatory effects of individual herbs in various taste groups. A) Summary of the activating (A1) and inhibitory (A2) effects of "sweet" herbs. B) Summary of the activating (B1) and inhibitory (B2) effects of "bitter" herbs. C) Summary of the activating (C1) and inhibitory (C2) effects of "pungent" herbs. Activation and inhibition were measured as described in Fig. 6. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (one-sample Student's t-test).

4. Discussion

In traditional Chinese medicine, the therapeutic effects of herbal medicines have been classified into distinct categories using different "taste" descriptors.[2] According to the traditional use of Chinese medicines, the taste descriptor provides a specific therapeutic description based on a long history of experience. This type of description usually applies to multi-dimensional pharmacological effects in the human body as a whole. DL is a systematic measurement that may be suitable for determining an herb's ethnopharmacological effect based on the taste descriptor. Here, we found that herbal materials in the same "taste" group generally produce similar DL curves. Interestingly, some herbs with the same taste descriptor (e.g., Codonopsis and Ginseng) can be interchangeable in some prescriptions for clinical applications.[10,27,28] In addition, the therapeutic effects of herbal medicines have been attributed to bioactive compounds,[29] and herbs within the same descriptor category can have very different profiles of bioactive compounds, as reflected in the Traditional Chinese Medicine Database (http://tcm.cmu.edu.tw/zh-tw/index.php). Moreover, the therapeutic effects of herbal medicines are not simply related to the identified bioactive compounds, as other chemical components within herbal medicines can combine synergistically with bioactive compounds to increase overall therapeutic efficacy.[30] Therefore, measuring an herb's DL signature may provide information regarding the biological characteristics and thus the specific therapeutic property indicated by the taste descriptor.

In principle, the molecular absorption of excitation energy defines the dynamics of the subsequent DL emission.[14,18] Changes in a compound's DL signature can be due to conformational changes in the cellular macromolecules, including proteins and nucleic acids.[31] Because both the primary and secondary metabolites in plants can interact with proteins and the cell surface by forming hydrogen bonds,[29] the molecular conformation of herbal cells can be changed, resulting in complex interactions between molecules. These interactions can affect the radiant (i.e.,

resonance) transfer of energy from one excited molecule to another, causing a change in the plant's DL dynamics.[31] Our analysis indicates that DL parameters can be used to identify herbs with a "sweet" descriptor. Since sweet taste can be derived from different types of compounds such as aldehydes, ketones, and sugar alcohols. The relationship between DL parameters and the intrinsic structure of "sweet" herbs needs to be explored further. This is also true with respect to the difference between herbs in the "sweet" category and herbs in the other "taste" categories. In addition, the finding that some herbs were misclassified in the PCA analysis may indicate the presence of other factors, other unknown mechanisms in DL, and/or the number of plant materials that we tested.

Another method for characterizing "taste" is to measure the biological response *in vivo*. However, this approach is currently limited. We therefore used an *in vitro* cell system to support our DL results. Herbal medicines with different taste descriptors have been reported to yield different effects with respect to their antioxidant and anti-inflammatory properties.[32,33] Here, we studied the immunomodulatory effects of herbs using a DC-based assay with water extracts. It is important to note that polysaccharides were removed before the samples were applied to the cells. Polysaccharides are a primary metabolite generally present in plants,[34,35] and polysaccharides in herbs can cause a significant immunostimulatory effect by boosting the immune system.[36–42] In addition, because secondary metabolites are the principle bioactive constituents in herbal medicines,[29] we focused on immunomodulatory responses induced by herbal water extracts without the influence of polysaccharides.

DCs are commonly used as a model cell system for antigen-presenting cells, which are activated to initiate an adaptive immune response.[43] The expression of the cytokines TNFα and IL-6 plays an essential role in the activation of DCs,[20,44,45] and the secretion of TNFα and IL-6 is a hallmark feature of DC activation used to measure immunostimulatory effects.[20,44] TNFα and IL-6 are tightly coupled, as

TNFα induces IL-6 production; therefore, TNFα is often used as an early marker of an immune response.[46] In addition, secretion of high levels of TNFα can harm the immune system, and when combined with other cytokines such as IL-6, pathological damage can occur.[47] However, in our study we measured the secretion of TNFα and IL-6 in response to LPS stimulation primarily to observe the ability of herbal medicines to suppress the immune response.

The "sweet" herbal materials tested here had an immunostimulatory effect corresponding to their traditional tonification action. The immunostimulatory effect in the "sweet" group differed significantly from the other groups; specifically, Ginseng radix et rhizoma rubra (S15) had both activating and suppressing effects on the immune response. Interestingly, this ambiguity regarding the effects of ginseng extract in vascular pathophysiology has been reported previously[48] and is consistent with our results. The immune response elicited by Codonopsis radix (S4) was similar to the immune response elicited by Ginseng radix et rhizoma rubra (S15). In traditional Chinese medicine, Ginseng and Codonopsis have a similar clinical action ("Qi tonifying") and have been categorized into the same group.[27] In addition, Codonopsis is usually substituted for Ginseng, although it has a weaker pharmacological effect in some applications in clinical practice.[27,28] This difference in pharmacological strength is supported by our results, which revealed differences in TNFα secretion between Codonopsis and Ginseng. In the presence of LPS, Astragali radix (S1) both increased TNFα secretion and inhibited IL-6 secretion; the latter effect is consistent with a report that a water extract of Astragali radix inhibits LPS-induced IL-6 release in human amniotic cells.[49] Several other studies indicate that polysaccharides derived from Astragali radix reduce LPS-induced TNFα release in various cell cultures.[50–52] Here, we found that a water extract of Astragali radix (without polysaccharides) increased the secretion of LPS-induced TNFα in DC cells, possibly indicating an opposing active principle in Astragali radix, similar to reports regarding ginseng.[48]

According to traditional Chinese medicine, the typical ethnopharmacological effects of "bitter" herbs are heat-cleansing.[9] Herbs with heat-cleansing effects are often used to treat infection, as well as to decrease tissue damage following inflammation.[53] Inflammation is a key component of autoimmune diseases such as rheumatoid arthritis and diabetes,[54,55] and increased secretion of TNFα and IL-6 are important inflammatory markers.[56] In our study, the majority of "bitter" herbs tested inhibited the secretion of both TNFα and IL-6. This result indicates that these "bitter" herbal samples have an immunosuppressive function consistent with their traditional pharmacological effects. In addition, these "bitter" herbs are reported to reduce the levels of TNFα and/or IL-6 in various pathological states.[57–60] In this respect, it is worth noting that Rhei radix et rhizoma (B8, rhubarb) significantly increased TNFα secretion and significant inhibited IL-6 secretion, thereby presenting opposing immunomodulatory effects. This finding may indicate that the immunosuppressive effects of rhubarb occur in a later stage in the immune response. On the other hand, Kounsar et al. reported that an ethyl acetate extract of rhubarb (*Rheum emodi* Wall. ex Meissn) increases the secretion of TNFα, thereby enhancing the immune response,[61] which is consistent with our results.

According to the principles of traditional Chinese medicine, "pungent" herbs can promote health by removing and expelling pathogenic factors such as heat, dampness, wind, and cold,[4] pathogenic factors that are closely related to diseases associated with inflammation.[62] Although both "pungent" and "bitter" herbs can reduce harmful factors, their pharmacological actions are not interchangeable.[4] The "pungent" herbal materials tested here inhibited both TNFα and IL-6 secretion, which is similar to the "bitter" herbal materials tested (although the effect was stronger for the "pungent" herbs). Similar results regarding these two herb groups were found in a study of NO production-based anti-inflammatory effects using herbal ethanol extracts.[33] In their study, the authors found that "pungent" herbs were slightly stronger anti-inflammatory agents than "bitter" herbs.[33] Thus, their results

are consistent with our findings and suggest that the Chinese herbal property of "taste" can be used to predict an herb's therapeutic effects.

The results regarding the immunomodulatory effects based on our DC assay are partially in agreement with our DL measurements, however, cell-based assay cannot fully represent the systemic response of herbs in the human body. Therefore, in this study, the use of DCs-based immunomodulatory assays is used to support the principle of herbal "taste" properties in TCM only, but supports—albeit indirectly—the results obtained from the holistic DL measurements. Thus, it is not meant to correlate DL results with specific immune responses (e.g., opposing effects in S4, S15 and B8) in DCs using herbal extracts. For further studies on correlations between herbal "taste" properties, therapeutic effects and DL characteristics, a total system-based model (e.g., zebrafish or other animal models) may provide a suitable option for future studies.

5. Conclusions

An interesting study discussed common pharmacological activity between different compounds such as ibuprofen and oleocanthal and noted their similarity regarding taste.[63] This approach is similar to the concept of "taste" in traditional Chinese medicine. The concept of herbal taste originates from traditional ethnopharmacological medical practices. The key challenge is to characterize and standardize herbal taste as a qualitative and quantitative predictor of pharmacological activity. Here, we present data supporting the use of DL to perform a systems level measure of an herb's pharmacological activity. In addition, we report the first study combining DL measurements with a cell-based assay in order to investigate herbal taste properties with respect to biological effects. Several main conclusions can be drawn from our study.

First, the parameters of the DL profiles were able to accurately discriminate the "sweet" category from other taste categories. Second, the results of our DC-based

immunomodulatory assay support the results obtained from our DL measurements. It is worth noting that DC-based assay is sensitive to LPS, therefore, possible contamination of herbal samples should be avoided. Finally, the results of this study may provide a suitable foundation for follow-up studies. For example, future studies can include a larger number of samples, more herbal taste categories, and/or more herbal plant components. In addition, additional platforms for screening the activity of herbal materials and the *in vivo* biological response should be performed in order to support the DL data using a large number of herbal materials. In this respect, a total system-based model (e.g., zebrafish) may provide valuable information to support the systematic observation of taste based on DL data. Moreover, improvements and developments of additional DL parameters and DL spectral analyses may provide a clearer distinction between different "taste" herbs such as "bitter" and "pungent" herbs. In conclusion, evidence-based interpretation of traditional herbal pharmacological effects is important for exploring the multi-dimensional pharmacological effects of herbs and for providing quality control.[64] DL is a robust, new technique for studying the ethnopharmacological effects of Chinese herbal medicines, thereby facilitating the move toward personalized healthcare.

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Supplementary Figure

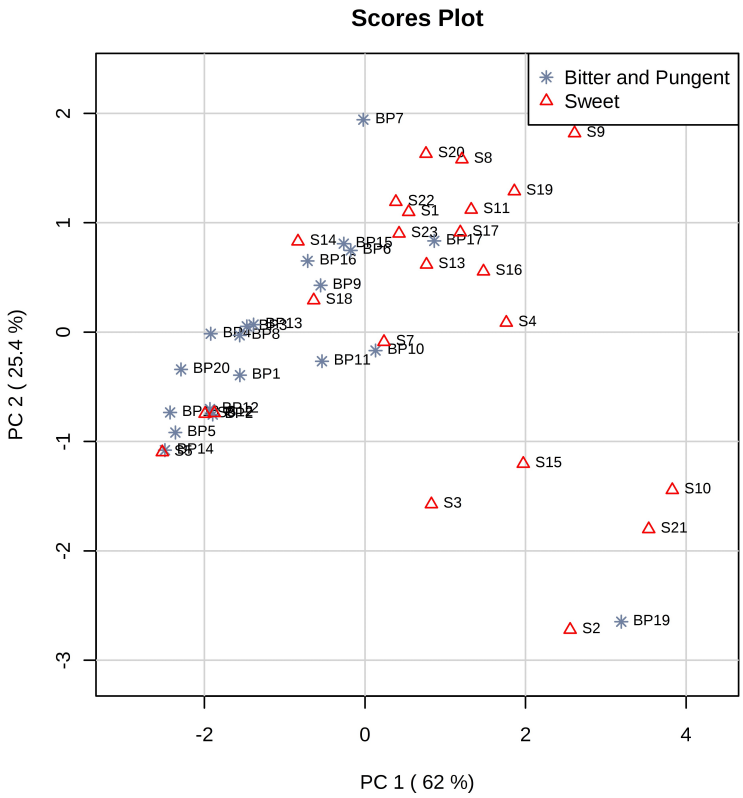

Fig. S1 PCA scores of the DL properties measured for “sweet” herbs and “bitter & pungent” herbs. Each symbol represents an individual herbal material with the corresponding ID number (see Table 1).