

## EXPERIMENTAL STUDY

## Oxalate contents of commonly used Chinese medicinal herbs

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**James Huang, Chris Huang**, University of California at Berkeley, Berkeley 94720, United States of America**Michael Liebman**, Department of Family and Consumer Sciences, University of Wyoming, Laramie 82072, United States of America**Correspondence to: Prof. Michael Liebman**, Department of Family and Consumer Sciences, University of Wyoming, Laramie 82072, United States of America. Liebman@uwyo.edu**Telephone:** +1-307-399-9519; +1-307-766-5597**Accepted:** October 16, 2014**Abstract****OBJECTIVE:** To assess the total and soluble oxalate contents of commonly used Chinese medicinal herbs.**METHODS:** Twenty-two Chinese medicinal herbs were extracted in both acid and water prior to determination of total and soluble oxalate, respectively. Oxalate was assayed in herbal extracts using a well-established enzymatic procedure.**RESULTS:** Among the 22 medicinal herbs, there was significant variation in oxalate content; *Houttuynia cordata* contained the highest amount of soluble oxalate (2146 mg/100 g) and *Selaginella doederleinii* contained the lowest amount (71 mg/100 g).**CONCLUSION:** The results indicated that different Chinese medicinal herbs, even from the same family, contain significantly different amounts of oxalate. In susceptible individuals, the use of medicinal herbs with the highest oxalate contents could increase risk of kidney stone formation.**Key words:** Drugs, Chinese herbal; Oxalates; Kidney calculi**INTRODUCTION**

Oxalate is a naturally occurring substance found in plants and in the human body.<sup>1</sup> In chemical terms, oxalate belongs to a group of molecules called organic acids. Certain body tissues routinely convert other substances into oxalate, which is an end product of human metabolism. For example, vitamin C can be converted into oxalate. In addition to its endogenous synthesis, oxalate can also be absorbed into the body from various food sources.<sup>1</sup> For example, fruits and vegetables such as kiwi, olives, beet greens, parsley, rhubarb, spinach, and Swiss chard are foods high in oxalate; others include wheat bran, almonds, cashews, sesame seeds, and foods which contain cocoa powder<sup>2</sup> (Liebman, unpublished data). It is interesting to note that the leaves of a plant usually contain higher oxalate levels than its roots, stems, and stalks.

Several recent studies have reported the oxalate contents of a variety of foods.<sup>2,3</sup> Grain-based flours are moderate sources of oxalate, with values ranging from 37 mg/100 g for brown rice flour to 269 mg/100 g for buckwheat flour. The range of total oxalate for nuts is 42 to 469 mg/100 g and for legumes is 4 to 80 mg/100 g of cooked weight.<sup>2</sup> Plant tissues contain soluble oxalate sources such as sodium and potassium oxalate and insoluble oxalate salts such as calcium and magnesium oxalate.<sup>1</sup> The efficiency of oxalate absorption is an important determinant of whether the consumption of a particular food significantly increases urinary oxalate excretion. The type of oxalate salt present in food may be important because soluble oxalate appears to be more bio-available than insoluble oxalate.<sup>1,4</sup>

Chinese medicinal herbs have long been used for thousands of years to treat a variety of diseases. Each medicinal herb has its own specific function. Some medicinal herbs come from stems only and others are from both

leaves and stems. In the present study, we summarized the functions of 22 Chinese medicinal herbs that are commonly used by Chinese medical doctors to treat patients (where applicable, we indicate the comparable over-the-counter drug that can be purchased at a regular pharmacy. These 22 medicinal herbs are commonly used to cure a variety of problems, such as typical cough, head cold, pain, fever, skin diseases, high blood pressure, mucus, jaundice, diarrhea, poison ivy, and poison oak, and in some cases may be used to prevent or help treat cancer and heart disease. Chinese medicinal herbs are typically used in a 3:1 ratio; that is, patients cook herbs with three cups of water until one cup of the herbal water remains, which is then drunk. As food oxalate content is a predictor of urinary oxalate excretion, which in turn has been directly linked to kidney stone formation,<sup>1,5,6</sup> the objective of this study was to assess the total and soluble oxalate content of typical Chinese medicinal herbs. We assumed that different Chinese medicinal herbs from the same families would contain very different amounts of soluble and insoluble oxalate.

## METHODS

Twenty-two commonly used Chinese medicinal herbs were selected for this study, including *Perilla frutescens*, *Nelumbo nucifera*, *Hedyotis diffusa*, *Heliotropium peruvianum*, *Siegesbeckia orientalis*, *Glechoma hederacea*, *Eupatorium cannabinum*, *Scutellaria barbata*, *Orthosiphon spiralis*, *Houttuynia cordata*, *Selaginella doederleinii*, *Plantago asiatica*, *Eclipta prostrata* (Linn.), *Leonurus artemisia*, *Folium Eriobotryae*, *Agastache*, *Schizonepeta*, *Folium Isatidis*, *Hypericum sampsonii*, *Taraxacum officinale*, *Artemisia indica*, and *Mentha arvensis*. These medicinal herbs were obtained from Hung Kuo Shin Medicinal Herb, Inc. (Taiwan, China).

The 22 Chinese medicinal herbs were first ground to a fine power using a coffee grinder. Then, 0.5 g of ground, dry herb was placed into a 250-mL flask; 50 mL of HCL was then added to one set of weighed samples and 50 mL of distilled de-ionized water added to another set of samples. Extraction in acid yielded an estimate of total oxalate, whereas extraction in water yielded an estimate of soluble oxalate. Flasks were placed in an 80 °C shaking water bath for 30 min after which 50 mL of distilled ionized water was added to the solutions and mixed by swirling. Approximately 10 mL of the solutions were transferred into a 15-mL centrifuge tube. The samples were then centrifuged at approximately centrifugal force of  $1778 \times g$  for 10 min and filtered with Whatman filter paper #1 into storage containers. The oxalate contents of all samples were determined by an enzymatic procedure using an oxalate kit (Trinity Biotech, Jamestown, NY, USA). In this procedure, oxalate is oxidized to carbon dioxide and hydrogen perox-

ide by oxalate oxidase. The hydrogen peroxide then reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye that has an absorbance maximum at 590 nm. The following procedure was used:

Sample preparation: a 1.0-mL volume of herbal extracts was pipetted into labeled glass culture tubes. A 1.0-mL volume of sample diluent (containing Ethylenediaminetetraacetic acid (EDTA) and a 7.6 pH buffer) was pipetted into the glass culture tubes containing the herbal extract and mixed using a vortex. pH levels outside of the range 5.0-7.0 were adjusted to this range using 5.0 N NaOH. The diluted herbal extracts were poured into labeled sample purifier tubes (containing activated charcoal). Each tube was vortexed for a few seconds and then placed in a metal rack mounted on a shaker and mixed for 5 min. The tubes were then centrifuged for 10 min at approximately a centrifugal force of  $1178 \times g$ . Using a Pasteur pipette, an aliquot of the clear supernatants (at least 100  $\mu$ L) was transferred from the purifier tubes into labeled microcentrifuge vials.

Oxalate determination: reagents A and B were allowed to reach room temperature. Reagent A contained DMAB (3.2 mmol/L), MBTH (0.22 mmol/L), and buffer (pH 3.1). Reagent B contained oxalate oxidase (Barley, 3000 u/L) and peroxidase (horseradish, 100 u/L). A 10- $\mu$ L volume of de-ionized water (for the blank), oxalate standard, or the herbal extract supernatants were pipetted to predesignated wells in the microplate. A total of 200  $\mu$ L of reagent A and 20  $\mu$ L of reagent B were pipetted into each well and mixed by gently tapping one side of the microplate. After 5 min, the absorbance was read at 590 nm using a microplate reader (Model EL 311, Bio-Tek Instruments, Winooski, Vermont).

## RESULTS

The functions of the 22 medicinal herbs are summarized in Table 1 and their average total and soluble oxalate contents are shown in Table 2. Among all 22 medicinal herbs, *Nelumbo nucifera* (Number 2 in the tables), *Houttuynia cordata* (10), and *Folium Eriobotryae* (15) contained the highest total oxalate levels. *Houttuynia cordata* and *Eclipta prostrata* Linn. (13) contained the highest soluble oxalate levels. *Houttuynia cordata* contained the highest total and soluble oxalate levels (3204 and 2146 mg/100 g, respectively), and *Selaginella doederleinii* (11) contained the lowest levels of total and soluble oxalate (165 and 71 mg/100 g, respectively).

## DISCUSSION

Twenty-two commonly used Chinese medicinal herbs were analyzed to determine their total and soluble oxalate contents. These herbs are used to treat typical prob-

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