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DIETARY SUPPLEMENTS

Variations in the Contents of Gingerols and Chromatographic Fingerprints of Ginger Root Extracts Prepared by Different Preparation Methods

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In the present study, an HPLC-DAD method was optimized for the quantitative determination of 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol in ginger extracts. A chromatographic fingerprinting method was also established to differentiate and evaluate the ginger extracts for bioactivity. Twenty-one extracts were prepared by methods differing in ginger type (fresh versus dried), solvent, and extraction methods. The ANOVA analysis showed the methods' influence on the mean extraction yields of gingerols increased in the order of: high pressure-high temperature (HP)>blender (BD)>low pressure (LP). The optimal solvent to extract gingerols was found to be 95% ethanol. The type of ginger used had significant effects on the content of gingerols, but its overall influence depended on the solvent used. In order to maximize the extraction efficiency of gingerols, a combination of dry ginger, 95% ethanol, and the HP extraction method should be employed. The chromatographic fingerprints were obtained to differentiate the unknown components from all ginger extracts. The similarity of the chromatographic fingerprints was used to evaluate the differences among all extracts. It can be concluded that the chromatographic fingerprints are able to ensure the stability of each extract and have some correlation with the observed bioactivity.

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Ginger (*Zingiber officinale*) is a perennial plant with narrow, bright green, grass-like leaves, and yellowish green flowers. Its rhizome is commonly processed as a spice, flavoring agent, and functional food, and has been used worldwide for generations. The medicinal properties attributed to ginger include anti-arthritic, antimigraine, anti-inflammatory, antithrombotic, antilipidemic, and antinausea (1–6). In recent years, animal and in vitro studies have shown ginger significantly lowered blood glucose, cholesterol, and other lipids in diabetic animals (7–10). It was also found that ginger had beneficial effects on free radicals, platelet aggregation, and hypertension (11–13).

Ginger is known to contain a rich source of potentially bioactive substances including pungent principles, which are mainly gingerols and their related dehydration products. The major components of ginger are gingerols (14–17), a mixture of homologs with 10, 12, and 14 carbons in the side chains, and which are designated 6-, 8-, and 10-gingerol, respectively. These ginger components have been shown to have a variety of pharmacological effects, including anti-inflammatory, anti-emetic, and cardiotonic properties (18–22).

Dietary supplements containing preparations of ginger roots or rhizomes (*Zingiber officinale*, Roscoe) are being used by consumers. However, clinical trials using dietary supplements have been carried out to evaluate ginger's anti-inflammatory or antiemetic properties with inconsistent results (23, 24). The lack of important chemical information subjects the cross-study comparisons of bioactivity variability difficult. It also hampers quality control efforts and causes difficulty in reproducing the results, and therefore, must be addressed.

Chemical standardization of these ginger extracts is needed for quality control and to facilitate the design of clinical trials and the evaluation of data from these studies. The quality assessment of herbal extracts has always been a challenging task due to the diversity of the multiple components existing in a complicated matrix. The chromatographic fingerprinting method has become one of the most frequently applied approaches, which can provide

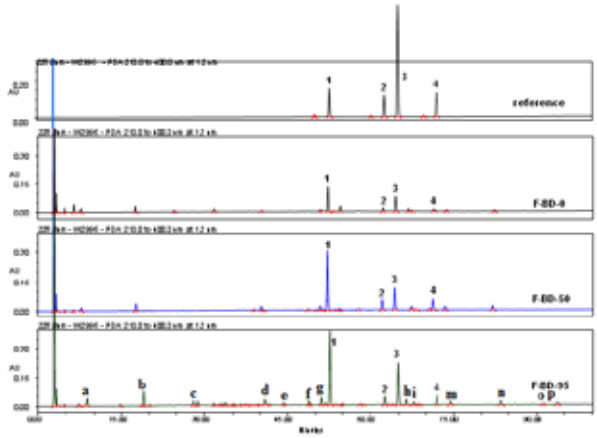

Figure 1. Representative chromatograms of the fresh ginger extracts prepared by the BD extraction method with three solvent regimens. Peaks 1, 2, 3, and 4 correspond to 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol, respectively. Letters a to p represent some characteristic fingerprint peaks. The number in each sample ID means that the extract was prepared by water, 50% ethanol, or 95% ethanol, respectively.

the whole profile of not only the marker compounds but also the unknown components. While this method has previously been used for the identification and quality control of many botanical medicines (25–28), the HPLC fingerprinting analysis of ginger and ginger extracts has not been reported.

The current study, for the first time, provides quantitative analyses of ginger's characteristic compounds and qualitative fingerprints of the ginger extracts that will allow meaningful bioactivity comparisons for future investigations. If chromatographic fingerprints and analyses were available, variations such as the preparation methods, sources, etc., could be taken into account for explaining the differences in observed bioactivities, and most importantly, allowing reproduction of the observed bioactivity.

In the present study, the commonly used ginger sample preparation methods were compared. These methods differed in ginger type (fresh or dry), solvents (water, saline water, or aqueous alcohol), and extraction methods: blender (BD), low pressure (LP), and high pressure-high temperature (HP). The

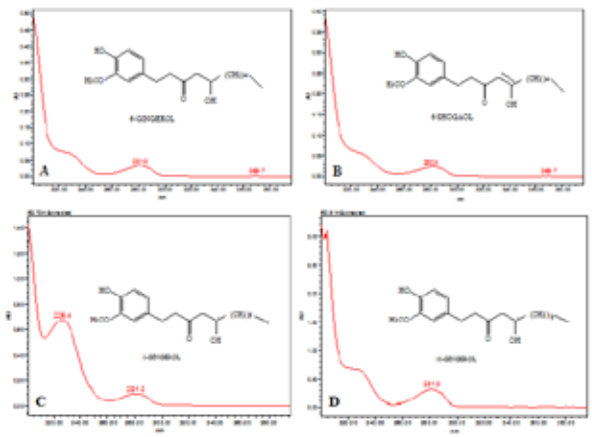

Figure 2. UV-spectrum of four representative gingerols; letters A, B, C, and D correspond to 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol, respectively.

gingerols are known for their characteristic aroma and are the active components in raw ginger. Therefore, it is conceivable that the yield of gingerols in the samples investigated may affect the outcomes of bioactivity testing among different investigations. As such, the yield of gingerols in response to the different extraction methods was used as a quantitative measurement for these samples. Fresh ginger seems to maintain rigid cellular structures, whereas dry ginger, a preferred method and form as a medicinal herbal ingredient, shows the rigid cell structures destroyed. The drying process also causes possible enzymatic activities that convert some constituents, especially gingerols, to different analogues. Thus, a comparison between fresh ginger and dry ginger was chosen.

Because gingerols are a class of compounds that exhibit poor water solubility, various solvent regimens were designed and compared using gingerols as quantitative indicators. Further, the resulting samples were analyzed quantitatively for several characteristic gingerols (6-, 8-, 10-gingerol, and 6-shogaol, and qualitatively for other unknown constituents. With this enhanced information, we are hoping future bioactivity results of ginger can be compared meaningfully among different research investigations, and most importantly, reproduced.

Experimental

Samples, Chemicals, and Equipment

The fresh ginger roots (*Zingiber officinale* Roscoe, family Zingiberaceae) were purchased from a local market and authenticated. Dry ginger roots were prepared by drying in an oven at 60°C until a constant weight was obtained.

The standard references of 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol were purchased from ChromaDex™ (Irvine, CA). HPLC-grade acetonitrile and water were obtained from Mallinckrodt (Phillipsburg, NJ). HPLC-grade phosphoric acid was provided by Fisher Scientific (Fair Lawn, NJ).

The stainless steel high-power blender (BD, model CB-15) was purchased from Waring Laboratory (Torrington, CT), the Timatic micro O/S LP extractor was purchased from Tecnolab (Rosi, Spello, Italy), and the accelerated solvent extractor (HP-ASE150) was purchased from DIONEX (Bannockburn, IL).

Extract Preparation

Ginger extracts were prepared with methods differing in ginger type (fresh or dry), solvents (water, 50% ethanol, and 95% ethanol), and extraction methods (BD, LP, and HP). The 2×3×3 treatment combinations, each with three replicates, were arranged to investigate the effect of three factors with different levels on the extraction efficiency of the four gingerols and the number of chromatographic peaks.

Fresh ginger (100 g) with skin attached was placed in a 4 L blender, with added water, 50% ethanol, or 95% ethanol, at a ratio of 1:10 (w/v), respectively. The water from the fresh ginger was calculated into the solvent used in the extraction process. The samples were blended intermittently at 6180 × *g* three times for 3 min each. After being centrifuged at 2060 × *g* for 10 min, the supernatants were filtered with a Whatman No. 4 filter paper, concentrated, and freeze-dried at 48°C for 48 h to the crude extract powders denoted as F-BD-0, F-BD-50, and F-BD-95, corresponding to the treatments of fresh ginger, blending

Table 1. Extraction recovery rates of four tested gingerols in ginger extracts (mean ± SE; *n* = 3)

Compounds	Level	Original content, μg	Spiked, μg	Detected, μg	Recovery, %[a]	RSD, %
6-gingerol	Low	146.2 ± 2.21	152.0	296.1 ± 2.00	98.6 ± 0.013	2.27
	Medium	288.3 ± 2.00	320.0	609.9 ± 3.27	100.5 ± 0.004	0.76
	High	434.1 ± 13.6	630.5	1056.7 ± 14.2	98.8 ± 0.140	0.32
6-shogaol	Low	28.89 ± 1.31	33.3	61.1 ± 0.82	96.6 ± 0.004	1.65
	Medium	55.69 ± 1.54	56.7	112.6 ± 2.09	100.3 ± 0.013	2.20
	High	87.4 ± 2.73	280.0	372.9 ± 2.06	101.9 ± 0.29	0.64
8-gingerol	Low	55.53 ± 1.18	63.8	120.1 ± 2.37	101.2 ± 0.019	3.20
	Medium	114.3 ± 1.97	111.7	227.5 ± 1.87	101.3 ± 0.001	0.20
	High	169.3 ± 5.29	392.5	552.1 ± 4.89	97.5 ± 0.210	0.49
10-gingerol	Low	35.4 ± 1.49	38.3	72.6 ± 1.23	97.1 ± 0.007	1.19
	Medium	67.8 ± 1.26	66.7	133.9 ± 1.97	99.1 ± 0.013	2.21
	High	101.0 ± 4.35	295.0	390.7 ± 3.52	97.9 ± 0.410	0.93

[a] Calculated as the equation= {(amount detected–original amount)/amount spiked} ×100%.

extracted by water, 50% ethanol, and 95% ethanol, respectively. The dry ginger (8.2 g obtained from 100 g fresh ginger) was extracted as described above for all treatments and was denoted by the prefix "D."

Fresh ginger (100 g) was cut into small pieces with skin attached and placed in an LP extractor. Water, 50% ethanol, or 95% ethanol, at a water–ethanol ratio of 1 + 10 (w/v) was added, respectively. The amount of solvent used in the extraction process was based on the initial water content from the fresh ginger samples. These samples denoted as F-LP-0, F-LP-50, and F-LP-95, were extracted under 586 kPa pressure for 69 min and processed as described above.

Fresh ginger (100 g) was cut into small pieces with skin attached and placed in a 34 mL stainless-steel extraction vessel of the accelerated solvent extractor (HP). Appropriate volumes of water, 50% ethanol, or 95% ethanol, were calculated automatically by the instrument. The water from the fresh ginger was calculated into the solvent used in extraction process. These samples denoted as F-HP-0, F-HP-50, and F-HP-95, were each extracted twice at 90°C under 11 mPa pressure for 15 min and processed as described above. In addition to the above treatments, three separate samples using the same batch of fresh ginger were also prepared. The Al-Amin method (23) was used to prepare the extract denoted as ZIO-Al-Amin, basically using saline to extract fresh ginger at a ratio of 1 + 2, w/v at room temperature. The Han extract was prepared following the Han method (24) and was denoted as ZIO-Han, which used water to extract fresh ginger at a ratio of 1 + 10, w/v at room temperature. The third extract was prepared by our own lab and used boiling water. The fresh ginger was cut into small pieces and was added to deionized water at a ratio of 1 + 10, w/v in a 2 L Erlenmeyer flask. The liquid was brought to and maintained at boiling for 30 min. The supernatant was then removed, filtered with a Whatman No. 4 filter paper, concentrated and freeze-dried to a crude extract powder denoted as ZIO-Own. Because these three methods had shown bioactivity, they were used as the control extracts for the purpose of comparisons with the other 18 preparation methods above.

Preparation of Standard Solutions

Four gingerols, 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol, were accurately weighed with their bottles, and an appropriate amount of HPLC-grade methanol was added. The solutions were transferred into 10 mL volumetric flasks and brought to the desired volume with methanol. These solutions were stored at –4°C as the stock standard solutions, which contained 0.97 mg/mL of 6-gingerol, 1.12 mg/mL of 6-shogaol, 1.57 mg/mL of 8-gingerol, and 1.18 mg/mL of 10-gingerol, respectively. Five milliliters of each of the four stock solutions were subsequently transferred into a 10 mL volumetric flask; the methanol was evaporated under nitrogen gas for one stock

Table 2. Effects of ginger type and extraction solvent on the contents of gingerols[a]

		Content % (*w/w*)				
Solvent	Type	6-gingerol	6-shogaol	8-gingerol	10-gingerol	Gingerols[b]
Water	Fresh	1.06 ± 0.30	0.13 ± 0.04	0.11 ± 0.05	0.06 ± 0.03	1.35 ± 0.36[d]
	Dry	0.93 ± 0.19	0.09 ± 0.02	0.10 ± 0.04	0.06 ± 0.03	1.15 ± 0.39[d]
50% Ethanol	Fresh	1.80 ± 0.54	0.40 ± 0.11	0.43 ± 0.15	0.48 ± 0.15	2.81 ± 0.82[c]
	Dry	1.71 ± 0.42	0.31 ± 0.08	0.45 ± 0.09	0.41 ± 0.17	2.63 ± 0.64[c]
95% Ethanol	Fresh	2.08 ± 0.54	0.42 ± 0.10	0.47 ± 0.12	0.50 ± 0.11	3.34 ± 0.81[b]
	Dry	4.05 ± 0.36	0.61 ± 0.12	0.68 ± 0.18	0.75 ± 0.11	5.29 ± 0.78[a]

[a] Mean ± SE; *n* = 9.

[b] Different letters following means indicate significant differences at $P \leq 0.05$.

Table 3. Effects of extraction method and extraction solvent on the contents of gingerols in the ginger root extracts[a]

Method	Solvent	Content (% w/w)				
		6-gingerol	6-shogaol	8-gingerol	10-gingerol	Gingerols[b]
LP	Water	0.16 ± 0.03	0.01 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.17 ± 0.03[e]
	50% Ethanol	0.25 ± 0.30	0.03 ± 0.01	0.08 ± 0.08	0.03 ± 0.01	0.27 ± 0.10[e]
	95% Ethanol	1.47 ± 0.30	0.20 ± 0.06	0.17 ± 0.15	0.27 ± 0.07	1.38 ± 0.51[d]
BD	Water	1.16 ± 0.30	0.20 ± 0.02	0.27 ± 0.04	0.17 ± 0.02	1.80 ± 0.16[d]
	50% Ethanol	1.66 ± 0.30	0.39 ± 0.03	0.35 ± 0.04	0.40 ± 0.01	2.80 ± 0.07[c]
	95% Ethanol	3.41 ± 0.41	0.50 ± 0.06	0.49 ± 0.08	0.54 ± 0.12	4.94 ± 0.40[b]
HP	Water	1.66 ± 0.22	0.12 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	1.84 ± 0.26[d]
	50% Ethanol	3.34 ± 0.39	0.65 ± 0.07	0.90 ± 0.04	0.90 ± 0.08	5.10 ± 0.53[b]
	95% Ethanol	4.30 ± 0.30	0.85 ± 0.09	1.07 ± 0.06	1.07 ± 0.15	6.62 ± 0.54[a]

[a] Mean ± SE; $n = 6$.

[b] Different letters following means indicate significant differences at $P \leq 0.05$.

solution before adding another until all four stock solutions were condensed to be within 10 mL and finally brought to volume. Serial dilutions were made to produce a series of concentrations of working standard solutions.

Preparation of Sample Solutions

All samples of the different extracts were freeze-dried, pulverized to a fine powder, and passed through a 100-mesh screen (<149 μm). One-tenth gram of each powdered sample was accurately weighed into a 50 mL Erlenmeyer flask and precisely 10 mL of 90% aqueous methanol was added. After the weight of the whole flask was recorded, the sample was sonicated for 20 min. The original solvent weight was restored after the sample was cooled to room temperature. This solution was filtered through a 0.2 μm syringe filter (Nalgene, Rochester, NY) prior to HPLC analysis.

HPLC–Photodiode Array (PDA) Analysis

The HPLC system used for all the analyses consisted of a Waters (Milford, MA) 600 pump, a Model No. 717 auto-sampler, and a Model No. 2996 UV-Vis PDA detector. HPLC separation was carried out on an Alltech Prevail C18 column (250 × 4.6 mm id; 5 μm) together with a YMC C18 guard column (7.5 × 4.6 mm id; 5 μm) with the column heater set at 25°C. The PDA detector collected spectra from 200–400 nm, with quantification performed at 228 nm. The mobile phase consisted of solvent A

Table 4. Effects of extraction method and solvent on the ratio of 6-gingerol to total gingerols in the ginger root extracts[a]

Type	Method	Solvent	6-gingerol	gingerols	6-gingerol/gingerols[b]
Fresh	BD	Water	0.95 ± 0.13	1.63 ± 0.07	0.59 ± 0.02[f]
		50% Ethanol	1.59 ± 0.11	2.71 ± 0.05	0.59 ± 0.01[f]
		95% Ethanol	2.53 ± 0.22	4.33 ± 0.17	0.59 ± 0.02[f]
	LP	Water	0.12 ± 0.03	0.12 ± 0.01	1.00 ± 0.00[a]
		50% Ethanol	0.09 ± 0.02	0.13 ± 0.01	0.82 ± 0.00[d]
		95% Ethanol	0.05 ± 0.01	0.27 ± 0.01	0.19 ± 0.04[g]
	HP	Water	1.37 ± 0.13	1.97 ± 0.05	0.91 ± 0.02[c]
		50% Ethanol	1.72 ± 0.13	2.87 ± 0.06	0.57 ± 0.02[f]
		95% Ethanol	4.30 ± 0.11	5.56 ± 0.13	0.63 ± 0.02[f]
Dry	BD	Water	0.20 ± 0.06	0.33 ± 0.03	0.69 ± 0.01[e]
		50% Ethanol	0.41 ± 0.05	0.95 ± 0.04	0.60 ± 0.02[f]
		95% Ethanol	2.90 ± 0.18	5.02 ± 0.09	0.78 ± 0.04[d]
	LP	Water	2.11 ± 0.21	2.29 ± 0.07	0.72 ± 0.09[d]
		50% Ethanol	3.70 ± 0.44	5.66 ± 0.16	0.60 ± 0.03[f]
		95% Ethanol	3.65 ± 0.08	5.44 ± 0.09	0.73 ± 0.01[d]
	HP	Water	1.22 ± 0.06	1.34 ± 0.02	0.93 ± 0.01[b]
		50% Ethanol	2.99 ± 0.66	4.54 ± 0.23	0.58 ± 0.02[f]
		95% Ethanol	4.96 ± 0.14	7.80 ± 0.06	0.56 ± 0.02[f]

[a] Mean ± SE; $n = 3$.

[b] Different letters following means indicate significant differences at $P \leq 0.05$.

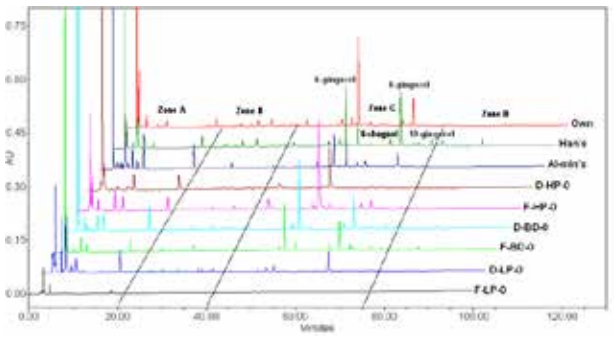

Figure 3. Chromatographic fingerprints of the ginger-water extracts prepared by the different extraction methods. F = fresh ginger, D = dried ginger, ZIO-Al-Amin = Al-Almin's method (23), ZIO-Han = Han's method (24), and ZIO-Own = boiling water method.

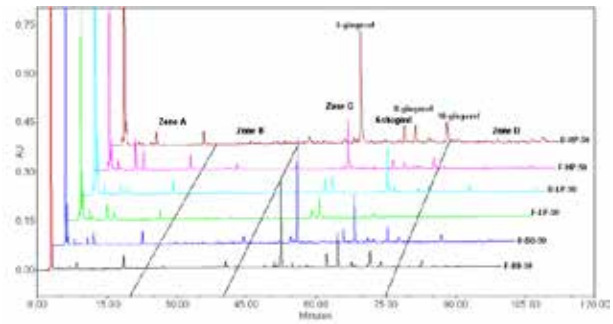

Figure 4. Chromatographic fingerprints of the ginger-50% ethanol extracts prepared by the different extraction methods. F = fresh ginger, D = dried ginger.

(0.17% phosphoric acid in acetonitrile) and solvent B (0.17% phosphoric acid in water). The gradient elution program was as follows: from 0 to 30 min, a linear change from 5 to 32%; from 30 to 90 min, a linear change from 32 to 92%; and finally, from 90 to 100 min, a linear change from 92 to 100%. The flow rate was set at 1.0 mL/min. The injection volume was 20 μL.

Method Validation

External standard calibrations were established at five data points covering the concentration range of each compound according to the levels estimated in the extracts. Working solutions were prepared by stepwise dilution of the stock solution with methanol. Triplicate analyses were performed for each concentration. The calibration curve was plotted as the peak area versus the concentration for each analyte. The linearity was evaluated by linear regression analysis calculated by the least squares regression method. The LOD and LOQ for each compound under the present chromatographic conditions were determined on the basis of responses at S/N ratios of three and ten, respectively.

To assess the precision of the method, the standard solution for each compound was injected six times for intraday precision. The interday precision was determined with the same standard solution but over 3 days. A recovery test was used to evaluate the accuracy of this method. Exact amounts of the four reference compounds were added to 0.05 g samples, which were then extracted and analyzed as described above.

Similarity Analysis

Similarity tests among the 21 treatments were performed based on the relative retention time and relative peak area, using the software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" (v. 2004A). The sample fingerprint matching was performed by using multipoint calibration mode based on the retention time and UV spectra. In this test, all 21 samples were examined to generate a mean chromatogram as the representative standard fingerprint, and the similarity of each chromatogram against this standard chromatogram was then calculated.

Statistical Analyses

Data were analyzed with Statistical Analysis System (SAS, Cary, NC). ANOVA analysis was performed to examine the interaction of different factors. Significance of all tests was set at $P \leq 0.05$.

Results and Discussion

Method Validation

The chromatograms of the extracts prepared by the BD method, which showed highest peak numbers among the 21 treatment samples, were chosen as the representative chromatograms (Figure 1). It was found that the selection of the UV detection wavelength and the resolution of each peak not only satisfied the quantitative analysis of the four gingerol compounds but also provided a more informative HPLC chromatogram displaying robust and balanced peaks. That is, it provided a comprehensive way to assess the quality of these extracts from both angles of marker compounds (gingerols) and non-marker components.

The peaks of the four gingerols in all chromatograms were identified by HPLC-PDA, based on their retention times, UV absorption spectra, and comparison with the available references. The UV spectra of the four gingerols are shown in Figure 2. It was found that the spectra of these four gingerols looked similar, there were two maximum absorption peaks observed at about 225 and 280 nm. The peaks of the four gingerols had the same PDA spectra, while other peaks with retention times beyond 40 min, showed similar spectra as the four representative gingerols in chromatographic fingerprints. These results illustrate that these peaks, numbered from d to p in the chromatographic fingerprint (Figure 1), can be identified as gingerols.

The standard curves for the tested components were linear over the studied concentration ranges with correlation coefficients ≥0.999. The LOD and LOQ values were 0.146, 0.336, 0.228, and 0.354 ng/mL and 0.487, 1.12, 0.76, and 1.18 ng/mL, for compounds 1–4, respectively. The RSDs of the intraday and interday precisions were found to be lower than 3.0%. The recoveries of compounds 1–4 were determined by the addition of a standard solution mixture at a concentration close to what would be expected in the actual extract samples, and the mean

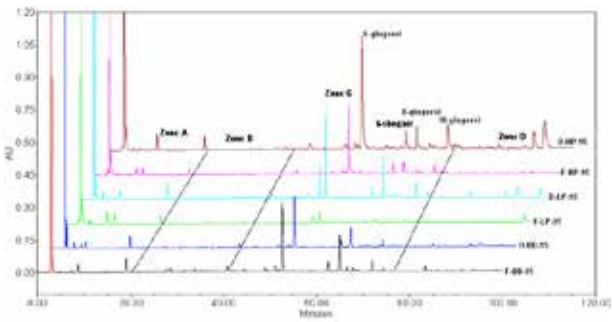

Figure 5. Chromatographic fingerprints of the ginger-95% ethanol extracts prepared by the different extraction methods. F = fresh ginger, D = dried ginger.

recovery rate was found to be in the range between 97.52 and 101.96%, with satisfactory RSDs in the range between 0.3 and 0.6% (Table 1).

Determination of Influential Factors in the Preparation Methods

The factorial arrangement with different levels was analyzed by using ANOVA to examine the main effects and their interactions on the content of the four gingerols and the number of peaks observed in the chromatograms. The solvent, type of ginger, and extraction method each had a significant influence on the content of gingerols. However, the effect of the ginger type or extraction method significantly depended on the extraction solvent used. A three-way interaction was not significant ($P < 0.05$). The solvent was the single most influential factor for the content of gingerols among the 18 extracts. The three control samples were also compared for their gingerol contents by using one-way ANOVA analysis, and the results showed there was no significant difference in the contents of gingerols among the three control methods.

The amounts of gingerols extracted by different solvent regimens was in the order of 95% ethanol > 50% ethanol > water. The amounts of gingerols extracted by 95% ethanol were almost two-fold of those extracted by 50% ethanol, and 4.6-fold of those extracted by water (Table 2). Using the same solvent regimen, it was found that 95% ethanol extracted significantly more (1.6-fold) gingerols from the dried ginger than from fresh ginger, whereas 50% ethanol or water extracted equal amounts of gingerols regardless of ginger type, dried or fresh.

The highest content of gingerols was seen using the HP extraction method and the 95% ethanol regimen, reaching 6.62% (Table 3). On the other hand, the extract prepared by the LP extraction method and water had only 0.17% gingerols, the lowest, of any extract. The effect of solvent regimens on the content of gingerols largely depended on the extraction method used. In the HP extraction method, the content of gingerols drastically increased from the water extract to the 50% ethanol extract and then significantly, but less drastically, increased further in the 95% ethanol extract. In the BD extraction method, the effect of solvent regimens was significantly linear in that the higher the ethanol concentration the greater the content of gingerols in the extracts. In the LP extraction method, however, only the 95% ethanol extraction had significantly more gingerols, whereas the combination of 50% ethanol and water produced extracts with the same gingerol content.

Generally, the mean extraction yields of gingerols increased in the order of: HP>BD>LP. HP and BD procedures extracted almost the same amount of gingerols when using water, but far more (11-fold) than those extracted by LP. HP with 50% ethanol extracted 11- and 1.8-fold more than those in the LP and BD methods, respectively. Clearly, to have a ginger extract that was highest in gingerols, the combination of using dry ginger, 95% ethanol, and the HP extraction method proved to be the best preparation method for ginger-root extract based on the conditions of this investigation.

Contents of Individual Gingerols in the Resulting Extracts

In all 18 ginger extracts, with the exception of one, the content of 6-gingerol was found to be much higher than that of 6-shogaol, 8-gingerol, or 10-gingerol, indicating 6-gingerol was the main constituent of the gingerols. Among the gingerols, 6-gingerol constituted a minimum of 58% (Table 4). The exception and extremes were found with the extract prepared by the LP extraction method with fresh ginger, in which 10-gingerol rather than 6-gingerol (19%) accounted for 41% of the gingerols in the 95% ethanol regimen, whereas in the water extract it was nothing but 6-gingerol (100%). Because 6-gingerol was the dominant component in nearly all gingerols, it was chosen as a predominant component to examine extraction efficiencies associated with different extraction methods using the ratio of 6-gingerol to total gingerols. As Table 4 shows, the three-way interaction was significant. There was no significant difference in the ratios among the three solvent regimens when using the same BD extraction method and fresh ginger. However, when using the LP extraction method, the solvent regimens had a significant influence on the ratios of 6-gingerol to total gingerols, ranging from 19 to 100%. Subsequently, when using the HP extraction method, the water extract had a significantly higher ratio of 6-gingerol to total gingerols than the equal 50 and 95% ethanol extracts.

The influence of extraction methods was different when dry ginger was used. In the BD extraction method, three solvent

Table 5. Similarity analyses among the chromatograms of 21 ginger extracts against a mean reference chromatogram[a]

Extract treatment	Similarity	Treatment	Similarity
D-BD-0	0.711	F-BD-0	0.394
D-BD-50	0.681	F-BD-50	0.469
D-BD-95	0.712	F-BD-95	0.716
D-HP-0	0.378	F-HP-0	0.742
D-HP-50	0.802	F-HP-50	0.810
D-HP-95	0.633	F-HP-95	0.709
D-LP-0	0.474	F-LP-0	0.114
D-LP-50	0.504	F-LP-50	0.184
D-LP-95	0.632	F-LP-95	0.716
ZIO-Al-Amin	0.376	ZIO-Own	0.449
ZIO-Han	0.492	Reference	1.000

[a] Software used: "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine" (v 2004A).

regimens resulted in extracts varying significantly in the ratio of 6-gingerol to total gingerols from 60 to 78%. In the LP method, water and 95% ethanol did not differ in the effect on the ratio, but 50% ethanol significantly lowered the ratio compared to the water and 95% ethanol. In the HP method using water, gingerols were composed of predominantly 6-gingerol (93%), whereas in the 50 and 95% ethanol extracts, their level was no more than about 60%. Overall, the water extract using the HP extraction method for dry ginger or the LP method for fresh ginger had the highest 6-gingerol ratios of 93 to 100%. However, the former had 11-fold higher total gingerol contents than the latter. The exceptional 95% ethanol extract using the LP extraction method and fresh ginger was the lowest in the 6-gingerol ratio with 10-gingerol (41%) being the major form of gingerols.

The Chromatographic Fingerprinting Analysis

In addition to the quantitative measurements of the four known gingerols prepared differently from each extract, a new HPLC chromatographic fingerprint was developed for each extract to trace the variation of other unknown components. To define and number the unknown peaks, the minimum peak area was set at 30 000 mAU, and the minimum peak height was set at 10 000 mAU. All the peaks were automatically integrated by the Empower II software. According to the cluster of peaks, the entire fingerprint was divided into four retention time zones as follows: zone A (0–20 min), zone B (20–40 min), zone C (40–75 min), and zone D (75–100 min). Zone A mainly contained the polar components of ginger extracts, characteristic of water extracts. Zone B mainly contained medium-polar components of ginger extracts, and this region displayed the greatest number of common peaks among all extracts. Zone C consisted of primarily the characteristic gingerol components of ginger extracts. Zone D contained those components with the least polarity, as seen commonly in the 95% ethanol extracts. Except for the four gingerols, none of the peaks in these zones are known. However, extracts were differentiated based on the number and distribution of peaks in the chromatographic fingerprints as well as the relative retention times and the relative peak areas.

When examining the chromatograms by zones, it was found that the water extracts mostly varied in the composition of zone C. Among the three control extracts, which were all water extracts, the Han's method (cold extract) and our laboratories' own (boiled) extracts were nearly identical, whereas the Al-Amin extract was different (Figure 3). Except for one extract (D-HP-0), all of the other water extracts had more components in zones B and C. In either the 50% ethanol (Figure 4) or 95% ethanol extracts (Figure 5), compositions were nearly identical in zone A, slightly variable in zones B and D, but most noticeable in zone C, featuring the characteristic gingerol components.

To further quantitatively evaluate the similarity of all 21 extracts prepared by various methods, a software was used to calculate the similarity of the different extracts, a process which is recommended for use in the similarity analysis of Chinese medicine by the State Pharmacopoeia Commission of China and has been widely applied in chromatographic fingerprint analysis of Chinese medicinal products, as well as related products. The chromatograms of 21 samples were analyzed with the software to generate a reference chromatogram. The similarity of each sample as compared to the reference chromatogram was then calculated (Table 5).

Similarities among the 21 samples ranged from 0.114 (the least similar) to 0.810 (the most similar). Generally, the closer the similarity values in Table 5, the more similar these samples are. For example, among the three control samples, the boiled water extract ZIO-Own (0.449) was over 90% similar to the room temperature water extract ZIO-Han (0.492), whereas ZIO-Al-Amin (the saline extract) was less similar to the two water extracts. Among the 18 extracts, D-HP-0 and F-BD-0 were most similar to the control ZIO-Al-Amin, whereas D-LP-0 and F-BD-50 were most similar to ZIO-Han. Therefore, based on the similarity analyses, it is predicted that the D-HP-0 and F-BD-0 extracts would reproduce the bioactivity related to ZIO-Al-Amin, while D-HP-0 and F-BD-0 would reproduce the bioactivity of ZIO-Han.

Clearly, as this study has demonstrated, ginger extracts prepared from the same source but using different methods have different compositions and these sample variations will also cause bioactivity discrepancies. In the current study, gingerols varied from 0.12 to 7.80%, a 65-fold difference between the low and high extremes. At the same testing dose, e.g., 2% in the food, the amount of gingerols delivered would totally depend on how the extracts were prepared. The variations in bioactivity from the different extraction procedures would lead to different conclusions, when everything else is the same (e.g., the same model systems under evaluation). On the other hand, if gingerols were not active constituents but rather involved other components like those in Zones A and D, then the contents of these components would be relevant to observed bioactivity. Because so little is known about the main bioactive components in the ginger root extracts, variations in their chemical constituents are very likely factors influencing the outcomes of bioactivity testing like those in the Al-Almin and Han publications. If these researchers had provided chemistry information of their test ginger samples, it would have been possible to explain why ginger was effective in their studies. The current study, for the first time, provides quantitative analyses of ginger's characteristic compounds and qualitative fingerprints that will allow meaningful future comparisons of compounds derived from ginger. Needless to say, the bioactivity discrepancies may not be simply explained even by the same preparation method. This is because the ginger source may be different, and thus, its chemical composition also different. Other reasons may include the use of different testing models. Regardless of the reasons, it is fundamentally important to have chemical analyses performed to allow meaningful comparisons among samples, and most importantly, reproducibility.

There are several publications that have investigated extraction efficiency for gingerol-related compounds. Hu et al. (29) examined the potential of pressurized liquid extraction (PLE) of ginger with bioethanol–water as solvents. PLE with 70% bio-ethanol operated at 1500 psi and 100°C for 20 min. (static extraction time: 5 min) was recommended as the optimized extraction conditions, achieving 106.8, 109.3, and 108.0% yield of 6-, 8- and 10-gingerol relative to the yield of corresponding constituent obtained by 8 h Soxhlet extraction (using absolute ethanol as the extraction solvent). Catchpole et al. (30) determined the overall yield and extraction efficiency for selected pungent components of ginger extracted by using near-critical carbon dioxide, propane, and dimethyl ether on a laboratory scale. All solvents quantitatively extracted the gingerols from ginger.

Three extraction methods (HP, LP, and BD) were used to prepare the extracts in the present study. HP used conventional liquid solvents at elevated temperatures and pressures to increase

the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, while elevated pressure keeps the solvent below its boiling point, thus enabling rapid extractions. The HP method was proved by our previous study as the most efficient method among the three extraction methods. In contrast, the LP extraction method at low pressure and ambient temperature took a longer time than the HP method to obtain equal efficiency.

The BD extraction method was the most convenient among the three extraction methods yet with some unique and desirable results. It combined grinding with rapid extraction, simultaneously avoiding the need of reducing the material size prior to extractions, such as the HP and LP methods required. The components were seen to be readily transferred into the solvent from the pulverized material. The speed of the BD extraction method can get up to 22 000 rpm/min, leading to a significant reduction of the extraction time to 3 min/cycle.

The advantages of these three extraction techniques over other extraction approaches, in addition to reduced time and solvent cost, are a distinct constituent profile with significantly improved recovery of gingerols. The extracts prepared by these three approaches are more suitable for bioactivity evaluation tests than other extracts because of the reduced levels of organic solvents.

Conclusions

An HPLC-DAD method has been developed and validated for the quantitative determination of 6-gingerol, 6-shogoal, 8-gingerol, and 10-gingerol as well as fingerprinting analyses of other major components in ginger root extracts. Different preparation methods of the same ginger root materials resulted in 65-fold different extracts as measured by the contents of gingerols and 5.3-fold by the number of chromatographic peaks. Heat-drying fresh ginger root prior to extractions did not significantly degrade gingerols. Aqueous ginger root extracts had significantly lower gingerol content than aqueous ethanol extracts. High temperature and pressure conditions extracted significantly more gingerols than other methods used in this study. Therefore, to maximize the extraction of gingerols, a combination of dry ginger, 95% ethanol, and the HP extraction method should be used. These results demonstrate the drastic variations of gingerols and other constituents associated with different preparation methods and establish a chemical basis for explaining bioactivity discrepancies among different investigations.

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