Analysis of Chemical Properties of Edible and Medicinal Ginger by Metabolomics Approach

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1. Introduction

Traditional medicine embodies accumulation of knowledge, skills, and practices on the maintenance of health as well as the prevention, diagnosis, improvement, or treatment of physical and mental illness. World Health Organization reported that, even today, more than 80% of the world’s population utilizes traditional medicine for primary health care [1]. Such medicinal system prescribes a combination of herbal, animal, and mineral parts, collectively known as crude drug, whose core materials are derived from plants including seeds, berries, roots, leaves, bark, or flowers [2]. The chemical constituents of crude drug are therefore considered a “chemical system,” which consists of a complex mixture of primary and secondary metabolites such as saponins, flavonoids, and alkaloids. The system is represented as a matrix in which rows and columns represent natural species and their chemical ingredients, respectively. This matrix works on another matrix representing the human body system, in which rows and columns represent interactive biomolecules (e.g., genes, proteins, and metabolites) and their tissue distribution, respectively. Thus, the research on traditional medicine deals with this “system to system” methodology, instead of the “point to point” methodology of western medicines (e.g., one particular chemical and its receptor gene). To understand the total function of traditional medicine, the knowledge of the interactions between matrices representing “chemical system” and “body system” is crucial. The matrix representing the human body system has gradually been made clear through several omics approaches, whereas knowledge on chemical system is not enough since almost all studies were done based on “point to point” or “point to system” methodology. Thus, we are accumulating the knowledge on chemical system with metabolomics approach [3–6].

Following our previous report on a newly registered turmeric (Curcuma longa cv. Okinawa Ougon) [3], we recently here investigate the chemical system of ginger cultivars. Ginger, the rhizome of the plant Zingiber officinale
Roscoe, is widely used as a spice and herbal medicine for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation, and diabetes [7]. The genus Zingiber is distributed in tropical and subtropical Asia, Far East Asia, and Africa and is under cultivation mostly in India and China. The global consumption of ginger has been increasing rapidly, and the recent, growing demand for natural products as additives for functional food and beverages makes ginger an ideal candidate for development. Thus, attempts at crop improvement for ginger have been performed in order to increase the yield and enhance the concentration of its active constituents. Traditionally, crop improvement involves controlled crosses (hybridization) between selected cultivars with desirable properties.

The target of our metabolomic approach is three medicinal ginger types, “Shokyo” (dried rhizome of Z. officinale var. rubens), “Kankyo” (steamed and dried rhizome of Z. officinale var. rubens) from Kampo (traditional Japanese) medicine, and “Red ginger” (rhizome of Z. officinale var. rubra) from Indonesian traditional medicine (Jamu) [8,9], and two edible ginger types, “Shoga” (fresh rhizome of Z. officinale var. rubens) from Japan and “White ginger” (rhizome of Z. officinale var. amarum) from Indonesia. From the comparison of five cultivars, we evaluate a new cultivar, Z. officinale cv. Ogawa Umare or “Ogawa Umare” (OG), and show its effectiveness as crude drug. OG was recently registered in the Japanese Plant Variety Protection (Ministry of Agriculture, Forestry and Fisheries, Japan) [10] and is characterized by its bold rhizome (3 times bigger than ordinary ginger) and a more pungent taste than standard medicinal ginger. All assays were conducted in a metabolomics platform with LC-MS and our results are consistent with the ginger taste.

2. Experimental

2.1. Specimens. The specimens of OG and “Shoga” used in this study were obtained from an official breeder. Fresh rhizomes of OG and “Shoga” were sliced and air-dried. Two specimens of Indonesian ginger, “Red ginger” and “White ginger,” were purchased from Oryza Oil & the Fat Chemical Co., Ltd. (Nagoya, Japan). Two Japanese herbal medicines, “Shokyo” and “Kankyo,” were bought from Tochimoto Tenkaido (Osaka, Japan). All specimens were deposited in the Museum of Materia Medica, College of Pharmaceutical Science, Ritsumeikan University (RIN).

2.2. Analytical Instruments. LC-MS analyses were performed using a Shimadzu LC-IT-TOF mass spectrometer equipped with an ESI interface. The ESI parameters were as follows: source voltage 4.5 kV, capillary temperature 200°C, and nebulizer gas 1.5 L/min. The LC-MS mass spectrometer was operated in the negative ion mode, scanning from m/z 50 to 2000. In the LC-MS analysis, a Waters Atlantis T3 column (2.1 mm i.d. × 150 mm) was used and the column temperature was maintained at 40°C. The mobile phase was a binary eluent of (A) 0.1% HCOOH solution and (B) CH3CN under the following gradient conditions: 0–30 min linear gradient from 20% to 100% B, 30–40 min isocratic maintained at 100% B. The flow rate was 0.2 mL/min.

2.3. LC-MS Sample Preparation. Individual specimens were homogenized to a fine powder using a multibeads shocker (Model MB755U, Yusi Kikai Co., Osaka, Japan). Two grams of the fine powder was accurately weighted and extracted four times with 50 mL of methanol under reflux conditions for 30 min. After centrifugation, the methanol layers were combined and evaporated in vacuo to give an extract. The extract was dissolved in 10 mL of methanol and filtered through 0.2 µm Millipore filter (polytetrafluoroethylene (PTFE) filter). Two milliliters of this solution was injected into LC-MS.

2.4. Standard Samples and Reagents. The isolated compounds ([6]-gingerol, [6]-shogaol, [6]-gingerdial, and diacetoxy-[6]-gingerdial) were identified by comparing their 1H- and 13C-NMR spectra with those reported in the literature [11, 12]. All chemicals were of analytical grade, and chromatographic solvents were of HPLC grade.

2.5. Data Analysis. All statistical analyses were carried out using Pirouette software (GL Science Inc., Tokyo).

2.6. Cell Proliferation Assay. HT-29 human colon cancer cells were seeded in 96-well plates (1 × 105 cells/well). Cells were allowed to adhere to overnight culture and then treated with metabolites at the final concentration of 3–100 µM. After a 72 h incubation, cell viability was determined with a WST-1 reagent (DOJINDO, Kumamoto, Japan).

3. Results and Discussion

The major pungent principles of ginger are gingerols and shogaols (dehydrated form of gingerols). The conversion of gingerols to shogaols is favored at higher temperature [7], and shogaols show stronger activity than gingerols [13]. As Japanese “Shoga” contains lower amount shogaol than Chinese one, heat processing is used for the production of herbal medicines derived from ginger. In this study, the oleoresins and their derivatives such as gingerdiols, acetoxyl gingerdiols, and diacetoxy gingerdiols [7] were identified based on mass spectral fragmentation with high-resolution mass data (Table 1). The fragmentation processes for [6]-gingerol, [6]-shogaol, [6]-gingerdial, and diacetoxy-[6]-gingerdial were determined from their mass spectra shown in Figure I. [6]-Shogaol gave the (M+H)+ ion at m/z 277.1807, whereas [6]-gingerol did not provide the (M+H)+ ion and showed (M+Na)+ and (M+K)+ ions at m/z 317.1717 and 333.1469, respectively, together with [M+(H−)–H2O]+ ion at m/z 277.1798. [6]-Gingerdiol predominantly provided the [(M+H)–2H2O]+ ion at m/z 261.1849 together with the weak (M+H)+ and [(M+H)–H2O]+ ions. In the case of diacetoxy-[6]-gingerdial, intense signals for the [(M+H)–CH3COOH]+ and [(M+H)–2CH3COOH]+ ions were observed at m/z 321.2046 and 261.1843, respectively. Furthermore, three characteristic adduct ions, (M+NH3)+, (M+Na)+, and (M+K)+, were detected. These results indicate that gingerol, shogaol, and their related compounds could be annotated by ESI mass spectral patterns together with high-resolution mass data.

LC-MS chromatograms of OG and “Shoga” are shown in Figure 2. Intense peaks in the respective chromatogram...
Table 1: Compounds in ginger, their compositions, and expected weight of (M+H)⁺ ions.

| Compounds             | C  | H  | O  | MW     | [M+H]⁺ |
|-----------------------|----|----|----|--------|--------|
| [6]-Paradol           | 17 | 26 | 3  | 278.1882 | 279.1960 |
| [7]-Paradol           | 18 | 28 | 3  | 292.2038 | 293.2117 |
| [8]-Paradol           | 19 | 30 | 3  | 306.2195 | 307.2273 |
| [9]-Paradol           | 20 | 32 | 3  | 320.2351 | 321.2430 |
| [10]-Paradol          | 21 | 34 | 3  | 334.2508 | 335.2586 |
| [11]-Paradol          | 22 | 36 | 3  | 348.2664 | 349.2743 |
| [13]-Paradol          | 24 | 40 | 3  | 376.2977 | 377.3056 |
| Methyl [6]-paradol    | 18 | 28 | 3  | 292.2038 | 293.2117 |
| [4]-Gingerol          | 15 | 22 | 4  | 266.1518 | 267.1596 |
| [6]-Gingerol          | 17 | 26 | 4  | 294.1831 | 295.1909 |
| [7]-Gingerol          | 18 | 28 | 4  | 308.1988 | 309.2066 |
| [8]-Gingerol          | 19 | 30 | 4  | 322.2144 | 323.2222 |
| [10]-Gingerol         | 21 | 34 | 4  | 350.2457 | 351.2535 |
| Methyl [4]-gingerol   | 16 | 24 | 4  | 280.1675 | 281.1753 |
| Methyl [6]-gingerol   | 18 | 28 | 4  | 308.1988 | 309.2066 |
| [4]-Shogaol           | 15 | 20 | 3  | 248.1412 | 249.1491 |
| [6]-Shogaol           | 17 | 24 | 3  | 276.1725 | 277.1804 |
| [8]-Shogaol           | 19 | 28 | 3  | 304.2038 | 305.2117 |
| [10]-Shogaol          | 21 | 32 | 3  | 332.2351 | 333.2430 |
| Methyl [6]-shogaol    | 18 | 26 | 3  | 300.1882 | 301.1960 |
| Methyl [8]-shogaol    | 20 | 30 | 3  | 318.2195 | 319.2273 |
| Acetoxy-[4]-gingerol  | 17 | 24 | 5  | 308.1624 | 309.1702 |
| Acetoxy-[6]-gingerol  | 19 | 28 | 5  | 336.1937 | 337.2015 |
| Acetoxy-[8]-gingerol  | 21 | 32 | 5  | 364.2250 | 365.2328 |
| Acetoxy-[10]-gingerol | 23 | 36 | 5  | 392.2563 | 393.2641 |
| Methyl acetoxy-[6]-gingerol | 20 | 30 | 5  | 350.2093 | 351.2172 |
| 1-Dehydro-[3]-gingerdione | 14 | 16 | 4  | 248.1049 | 249.1127 |
| 1-Dehydro-[6]-gingerdione | 17 | 22 | 4  | 290.1518 | 291.1596 |
| 1-Dehydro-[8]-gingerdione | 19 | 26 | 4  | 318.1831 | 319.1909 |
| 1-Dehydro-[10]-gingerdione | 21 | 30 | 4  | 346.2144 | 347.2222 |
| [4]-Gingerdiol        | 15 | 24 | 4  | 268.1675 | 269.1753 |
| [6]-Gingerdiol        | 17 | 28 | 4  | 296.1988 | 297.2066 |
| [8]-Gingerdiol        | 19 | 32 | 4  | 324.2301 | 325.2379 |
| [10]-Gingerdiol       | 21 | 36 | 4  | 352.2614 | 353.2692 |
| 5-Acetoxy-[4]-gingerdiol | 17 | 26 | 5  | 310.1780 | 311.1859 |
| 5-Acetoxy-[6]-gingerdiol | 19 | 30 | 5  | 338.2093 | 339.2172 |
| 5-Acetoxy-[7]-gingerdiol | 20 | 32 | 5  | 352.2250 | 353.2328 |
| Methyl 5-acetoxy-[4]-gingerdiol | 18 | 28 | 5  | 324.1937 | 325.2015 |
| Methyl 5-acetoxy-[6]-gingerdiol | 20 | 32 | 5  | 352.2250 | 353.2328 |
| Diacetoxy-[4]-gingerdiol | 19 | 28 | 6  | 352.1886 | 353.1964 |
| Diacetoxy-[6]-gingerdiol | 21 | 32 | 6  | 380.2199 | 381.2277 |
| Methyl diacetoxy-[4]-gingerdiol | 20 | 30 | 6  | 366.2042 | 367.2121 |
| Methyl diacetoxy-[6]-gingerdiol | 22 | 34 | 6  | 394.2355 | 395.2434 |
| Methyl diacetoxy-[10]-gingerdiol | 26 | 42 | 6  | 450.2981 | 451.3060 |
Figure 1: Mass spectra of (a) [6]-shogaol, (b) [6]-gingerol, (c) [6]-gingerdiol, and (d) diacetoxy-[6]-gingerdiol.

Figure 2: LC-MS chromatograms of (a) OG and (b) "Shoga."
were annotated by detailed analysis of their mass spectral data. Comparison of the chromatographic data shows that OG contains larger amounts of diacetoxy-[6]-gingerdiol and methyl diacetoxy-[6]-gingerdiol than “Shoga.”

In order to clarify the medicinal properties of ginger, LC-MS chromatograms of the extracts of all six ginger types are shown in Figure 3. Although there are clear visual differences between the chromatograms of the upper three and lower three samples in Figure 3, this classification does not match their medicinal usage or tastes. For more unbiased interpretation and to reduce the dimensionality of the multivariate data, we analyzed the LC-MS chromatographic data using principal component analysis (PCA).

PCA is an unsupervised method of multivariate data analysis and is used for clarifying the characteristic properties of the metabolomic profiles of complex mixtures, such as plant extracts. The annotated peaks and relative intensities detected in the chromatograms of the extracts (Table 2) were normalized and subjected to the PCA analysis. In Figure 4, the PCA scores plot and loading plot were shown. The first two PCs accounted for 90.4% of total variance (PC1, 71.1%; PC2, 19.3%). The scores plot clearly indicated that the chemical content patterns of the medicinal and edible ginger were different. In the chemometric analysis, the peaks having big loading values could be considered as the makers strongly contributing to the classification of the samples by PCA. In the present results, “Shokyo” and “Kankyo” showed similar properties, which were higher concentrations of acetoxyl-[6]-gingerdial and diacetoxy-[6]-gingerdial. “Red ginger” was also characterized by its higher content
of acetylated compounds, but low methyl diacetoxy-\([6]\)-gingerdiol content. The new cultivar, OG, was also grouped with medicinal ginger. On the other hand, two edible ginger types (raw \(Z\). \textit{officinale} var. \textit{rubens} and \(Z\). \textit{officinale} var. \textit{amarum}) showed higher contents of \([10]\)-gingerol and lower contents of acetylated compounds. Although \(Z\). \textit{officinale} var. \textit{rubens} is used as the raw material in the production of Kampo medicine (“Shokyo” and “Kankyo”), only the most pungent fresh ginger is selected and utilized [14], which suggests the importance of shogaols and gingerols, the pungent and active constituents, for medicinal purpose [8]. So far, \([6]\)-gingerol and \([6]\)-shogaol were described as main bioactive constituents of ginger with “point to point” methodology [15–17], whereas \([6]\)-gingerol was reported to be metabolized to (3R,5S)- and (3S,5S)-6-gerdidiols in mice to induce cell death toward H-1299 cancer cells [11]. On the other hand, our metabolomics approach to chemical system of medicinal ginger is based on “system to system” methodology and has suggested the importance of acetylated compounds, diacetoxy-\([6]\)-gingerdiol. Thus, we examined the cytotoxicity of diacetoxy-\([6]\)-gingerdiol, a main constituent of OG. As shown in Figure 5, diacetoxy-\([6]\)-gingerdiol exhibited stronger cytotoxicity to HT-29 human colon cancer cells than \([6]\)-gingerol. These results should indicate the importance of acetylated compounds such as diacetoxy-\([6]\)-gingerdiol for the use as Kampo medicine and for the classification of medicinal and edible ginger. In addition, from the viewpoint of its chemical constituents and rhizome yield, OG has valuable properties as a new resource for the production of herbal medicines derived from ginger.

**Table 2:** Annotated peaks and relative intensities detected in the chromatograms of the ginger extracts.

| Compounds                  | Retention time (min) | Red ginger | OG       | Relative intensity |
|----------------------------|----------------------|------------|----------|--------------------|
| \([6]\)-Gingerdiol         | 20.13                | 6318188    | 3419547  | 35356476           |
| \([6]\)-Gingerol           | 20.93                | 167039972  | 46057215 | 20037041           |
| Methyl \([6]\)-gingerol    | 22.97                | 9115912    | 7474131  | 10056226           |
| 5-Acetoxy-\([6]\)-gingerdiol | 22.97              | 41701072   | 14320787 | 8371635            |
| Diacetoxy-\([4]\)-gingerdiol | 23.58              | 0          | 3764215  | 3927640            |
| \([8]\)-Gingerol           | 23.78                | 14502585   | 2161811  | 1983957            |
| \([8]\)-Gingerol           | 24.48                | 53406657   | 13470644 | 3991218            |
| Acetoxy-\([6]\)-gingerol   | 24.60                | 0          | 5066601  | 2597449            |
| Methyl 5-acetoxy-\([6]\)-Gingerdiol | 24.95        | 13575229   | 17357866 | 18372059           |
| \([6]\)-Shogaol            | 25.37                | 55671256   | 0        | 34992211           |
| Methyl \([6]\)-shogaol     | 26.33                | 17170183   | 7035201  | 5050693            |
| Methyl acetoxy-\([6]\)-gingerol | 26.33           | 11794505   | 1309059  | 1624192            |
| Diacetoxy-\([6]\)-gingerdiol | 26.70              | 36851432   | 11740397 | 88966249           |
| 1-Dehydro-\([6]\)-gingerdione | 27.13              | 9896109    | 8071086  | 63993719           |
| \([10]\)-Gingerdiol        | 27.21                | 18056089   | 1755611  | 1728210            |
| \([10]\)-Gingerol          | 27.79                | 31802196   | 19048130 | 5682838            |
| \([8]\)-Shogaol            | 28.62                | 20999783   | 0        | 7432153            |
| Methyl diacetoxy-\([6]\)-gingerdiol | 28.62       | 19086315   | 13667077 | 16641942           |
| \([10]\)-Shogaol           | 31.52                | 7237938    | 0        | 4268883            |

**Figure 5:** Cell viability of HT-29 human colon cancer cells after treatment with \([6]\)-shogaol, diacetoxy-\([6]\)-gingerdiol, \([6]\)-gingerol, and \([6]\)-gingerdiol.

### 4. Conclusion

Up to now, several studies reported on the contribution of \([6]\)-gingerol and \([6]\)-shogaol to many biological activities of ginger. Prasad and Tyagi summarized many molecular targets of the compounds [15]. However, medicinal activities of ginger are not attributable to only \([6]\)-gingerol and \([6]\)-shogaol. Their derivatives have been actively investigated for novel bioactivities such as antihaemolysis by longer chain oleoresins [18], quorum sensing inhibition by \([6]\)-azashogaol [19], and antiplatelet aggregation by \([6]\)-paradol [20]. Synergistic bioactivity of \([6]\)-gingerol with another metabolite is also reported [21]. Our observation that acetoxy derivatives are relatively abundant in medicinal ginger and
the compound possesses biological activities may provide additional clues to find more bioactivities of ginger. On the other hand, scarcity of [10]-gingerol, [12]-gingerol, or gingerdiols in both medicinal and edible ginger indicates that these bioactive components [22] play fewer roles in the medication of traditional medicines.

The molecular targets of the certain compounds have gradually been made clear through several omics approaches, whereas knowledge on chemical system is still limited. Integration of the knowledge of “chemical system” as described in this paper may help understand the action between “chemical system” and “body system” in traditional medicines.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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