The Effects of Microbubble Washing on Microbial Contamination and the Active Ingredient Contents of Crude Drugs

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Abstracts: The quality of crude drugs critically influences their effective and safe use. Because crude drugs are natural products, their improper handling can easily cause microbial contamination, which in turn affects their quality and endangers human health. This study investigated microbubble washing as a new decontamination method and studied its effect on the microbial load and main active ingredients of five different crude drugs. The total bacterial and coliform counts were assessed using Petrifilm plates. Liquid chromatography-mass spectrometry and high-performance liquid chromatography were performed to quantify the crude drugs' major constituents. The five crude drugs were selected to evaluate five classes of compounds: *Scopolia* rhizome for alkaloids; *Anemarrhena* rhizome for saponins; *Aralia* rhizome for diterpenes; peony root for monoterpene glycosides; and *Scutellaria* root for flavonoids. Because many crude drugs have structural features that hinder cleaning, some could not be sufficiently sterilized by microbubble washing alone. However, microbubbles with the addition of sodium hypochlorite significantly improved the sterilization efficacy. Conversely, even microbubble washing with sodium hypochlorite did not produce sufficient bactericidal effects in crude drugs with leaf-derived hair-like structures, such as *Anemarrhena* rhizome. In such cases, longer washing times and higher hypochlorite concentrations were implemented. No significant changes were observed in the major constituents of the five

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different crude drugs studied. The results of this study indicate that microbubbles can effectively reduce the microbial load of crude drugs without significantly impacting the primary constituent contents, with the advantage of short cleaning time and reduced water resource waste compared to simple water cleaning. Thus, microbubble washing offers a new method for the sterilization of crude drugs.

Keywords: *Microbubbles, Crude drugs, Microbial load, Active ingredients content*

1. Introduction

Herbal medicines of plant origin are contaminated with sand and soil at the time of harvest and also coexist with or are contaminated by various microorganisms. Residual precipitates reflect poor manufacturing of crude drugs as pharmaceuticals and significantly reduce their quality. In addition, the microbial load needs to be minimized during the production process to maintain standards of quality, safety, and efficacy to reduce health risks and damage (He et al. 2015; Okunlola et al. 2007; World Health Organization 2007). Therefore, the Japanese Pharmacopoeia strictly regulates the amount of residual sand and soil in herbal medicines of plant origin using the ash test, acid-insoluble ash test, and microbiological tests to ensure the quality of crude drugs as pharmaceutical products (Ministry of Health, Labour and Welfare 2021).

Various crude drug processing methods have been developed since ancient times. In the 5th century, the volume "Master Lei's Discourse on Drug Processing" (雷公炮炙論) summarized the crude drug processing methods of the time, significantly impacting crude drug processing approaches in subsequent periods (Chinese Text Project n.d. a). Moreover, Ming's Miao Xiyong (繆希雍) wrote the "Processing Methodology" (炮炙大法) and organized 17 processing methods (炮炙十七条) based on "Master Lei's Discourse on Drug Processing" (Chinese Text Project n.d. b). However, these processing methods were compiled with a focus on processing methods aimed at reducing the toxicity and improving the efficacy of each crude drug. A description of cleaning and processing methods using water can be found in "Ben Cao Meng Quan" (本草蒙筌) written by Chen Jiamo (陳嘉謨) in the 16th century (Ming dynasty) (Chinese Text Project n.d. c). This book describes three different processing methods using water for cleaning and other purposes, such as dampening (浸), soaking (泡), and washing for purification (洗之弗). The first two approaches could be considered to facilitate subsequent processing and reduce toxicity. Currently, these water-based processing methods for cleaning and other purposes have been further developed. The washing methods used are splashing (淋法) applied to soft crude drugs, such as the leaves and pericarp, and washing with clean water (淘洗法) applied to the underground parts. Washing should be performed in a short time to avoid the elution of the crude drug's active ingredients. Meanwhile, to soften crude drugs and reduce their poisonous components, soaking (泡法) or immersion in running water (漂法) are also used, both of which require prolonged water use. In any case, considering the recent increase in the amounts of crude drugs in use, their treatment generates a considerable amount of wastewater and imposes a burden on the environment (Huang et al. 2021). Therefore, safe, efficient, and environmentally friendly crude drug cleaning methods are urgently required.

In recent years, stricter hygiene standards have been imposed for crude drugs. Simple water cleaning has faced challenges in meeting sanitary requirements. Therefore, the industry often opts for combined sterilization methods to ensure crude drug quality. The sterilization methods that have been used include high-temperature steam, radiation (Ernawati et al. 2021), and chemicals, such as sulfur (Jiang et al. 2013) and ethylene oxide (Dehghani et al. 2009). However, chemical sterilization can alter the quality of crude drugs by leaving behind toxic residues, diminishing the potency

of active ingredients due to sulfur use (Jiang et al. 2013), or producing toxic byproducts, such as ethylene chlorohydrin and ethylene glycol (Ghisleni et al. 2016), due to ethylene oxide use.

Recent studies have reported the efficacy of microbubbles in disinfecting and sterilizing vegetables and fruits (Inatsu et al. 2011; Klintham et al. 2017; Li et al. 2021; Li et al. 2023; Ushida et al. 2017). Microbubbles exert their cleaning and disinfection effects physically and chemically (Jin et al. 2022; Sun et al. 2022; Zhang et al. 2021). Physical properties, such as a large specific surface area, increase the probability of contaminant attachment (Ishizaki et al. 2018), and jet impingement during bubble fracturing mechanically separates contaminants from the solid surface of an object (Ohl et al. 2006; Zhang et al. 2021). At the same time, microbubbles spontaneously generate hydroxyl radicals and other reactive oxygen species, which exert a strong oxidizing effect, consequently producing a bactericidal effect (Liu et al. 2016 a; Liu et al. 2016 b; Wang et al. 2018).

Intending to accumulate knowledge for the application of microbubble washing in the processing of crude drugs, this study examined the effects of microbubble washing on the bactericidal effect and active ingredients of crude drugs as a new cleaning method. Based on their structural characteristics and diversity of ingredients, we selected the following five crude drugs for testing: *Scopolia* rhizome, *Scutellaria* root, *Aralia* rhizome, *Anemarrhena* rhizome, and peony root.

2. Experimental Methods

(1) Materials

Baicalin, paeoniflorin, atropine sulfate hydrate, and scopolamine hydrobromide hydrate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Timosaponin AIII and kaurenoic acid were obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Anemarsaponin B was obtained from TargetMol Chemicals Inc. (Boston, MA, USA). Brucine dihydrate was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). 3M™ Petrifilm™ Aerobic Count (AC) Plates and 3M™ Petrifilm™ E. coli/Coliform Count (EC) Plates were purchased from 3M Microbiology Products (Saint Paul, MN, USA). Other analytical-grade chemicals and chromatographic solvents for liquid chromatography-mass spectrometry (LC-MS) were purchased from either FUJIFILM Wako Pure Chemical Corporation or Nacalai Tesque, Inc. (Kyoto, Japan).

(2) Plant Materials

The five crude drugs were cultivated at the medicinal botanical garden of Hokuriku University. The five crude drugs were identified as *Scopolia* rhizome, *Scutellaria* root, *Aralia* rhizome, *Anemarrhena* rhizome, and peony root by Dr. Yukio Kawata of Hokuriku University. The voucher specimens for these crude drugs were deposited at the Museum of Materia Medica, Ritsumeikan University.

(3) Crude Drug Processing

A K600 precision microbubble sterilizing machine (Kiyomizu Kougyoujyo Co., Ltd., Aichi, Japan) was used to produce microbubbles with and without 0.01% NaClO. The bubble generator sprays water at a flow rate of about 12 L/min through about 2.0–4.0 mm nozzles to generate microbubbles. The generation of microbubbles causes the water to assume a state of white turbidity and remain so for 10–30 minutes. In a previous study (Ushida et al. 2016), it was shown that

microbubbles less than 100 μm in diameter caused water to have a white turbidity and that persisted for more than 600 seconds. In the present study, white turbidity and its duration were observed, which were similar to those reported by Ushida et al. Based on these observations, the size range was estimated to be less than 100 μm. Microbubble generation stabilized before cleaning. Each crude drug was randomly divided into three treatment groups: The control group was washed with running water (rinsed twice for 5 min each time); the microbubble group, with running microbubbles (rinsed twice for 5 min each time); and the microbubble + 0.01% NaClO group, with running microbubbles containing 0.01% NaClO (rinsed twice for 5 min each time).

(4) Microbiological Tests

Commercially available AC plates were used for total bacterial detection, while EC plates were used to measure coliform content according to the manufacturer's instructions. Five grams (5 g) of crude drug treatments from each group were obtained using sterile scissors and tweezers, placed in a sterile sample bag, filled with 45 mL sterile phosphate buffer solution (PBS), and allowed to stand for 10 min. Subsequently, PBS was decanted from each sample bag and diluted 10-fold to make a sample solution for analysis. Next, 1 mL of each sample solution was inoculated onto the center of the plates, which were then incubated. Sample solutions were prepared in triplicate for each group. Plates for total bacterial assay were incubated at 34°C ± 1°C for 48 h. The coliform assay plates were incubated at 34°C ± 1°C for 24 h. After incubation, the number of bacterial colonies was counted.

(5) Standard Solution Preparation

Reference standards were precisely weighed, then dissolved in different solvents (scopolamine and atropine were dissolved in the mixture of acetonitrile and 0.05 mol/L potassium dihydrogen phosphate solution [containing 1% triethylamine, adjusted to pH 3.5 with phosphoric acid] [10:90, v/v], kaurenoic acid and baicalin in methanol, paeoniflorin in 50% methanol, and anemarsaponin B and timosaponin AIII in 90% methanol) to prepare standard stock solutions at a concentration of 1 mg/mL. The standard stock solutions were diluted to prepare calibration solutions to the required concentrations of 0.05, 0.10, and 0.50 mg/mL. Among them, anemarsaponin B exhibited concentrations of 0.01, 0.05, and 0.10 mg/mL, and timosaponin AIII was of 0.005, 0.01, and 0.05 mg/mL. All these solutions were filtered through a 0.45-μm polytetrafluoroethylene (PTFE) membrane and stored at 4℃ prior to analysis.

(6) Extract and Sample Solution Preparation

We dried the crude drugs at 50°C for 5 days, then weighed and powdered them using a TUBE-MILL 100 milling machine (Model C S004, IKA, Germany), and submitted them to extraction according to the Japanese Pharmacopoeia, 18th Edition (Ministry of Health, Labour and Welfare 2021) as follows.

Peony root: The peony root powders (500 mg) were precisely weighed and extracted with 50 mL of 50% methanol (v/v) under reflux conditions for 30 min, followed by cooling and filtering. The residues were supplemented with 50 mL of 50% methanol (v/v) and proceeded similarly. After extraction, all the filtrates of each sample were combined and supplemented with 50% methanol (v/v) to produce exactly 100 mL of sample solutions.

Scutellaria root: The *Scutellaria* root powders (500 mg or 100 mg) were precisely weighed and

extracted with 30 mL (for 500 mg of powders) or 6 mL (for 100 mg of powders) of 70% methanol (v/v) under reflux conditions for 30 min, followed by cooling, centrifugation and separation of the supernatant liquid. The residues were supplemented with 30 mL or 6 mL of 70% methanol (v/v) and shaken for 5 min, centrifuged, and separated from the supernatant liquid, and this procedure was repeated twice. All the extracts of each sample were combined and supplemented with 70% methanol (v/v) to produce exactly 100 mL (for 500 mg of powders) or 20 mL (for 100 mg of powders) of the solution, then we took exactly 2 mL of this solution and added 70% methanol (v/v) to produce exactly 20 mL of sample solutions.

Scopolia rhizome: The *Scopolia* rhizome powders (700 mg) were precisely weighed and moistened with 15 mL of 10% ammonia solution (v/v). Next, 25 mL of diethyl ether was added to the preparation and shaken for 15 min, then centrifuged and separated from the diethyl ether layer. Then 25 mL of the diethyl ether was added to the residues and processed in the same way as above, and this procedure was repeated twice. All the extracts of each sample were combined, and we removed the solvent. The residues were dissolved in 5 mL of the mobile phase, which produced a mixture of acetonitrile and 0.05 mol/L potassium dihydrogen phosphate solution (containing 1% triethylamine, adjusted to pH 3.5 with phosphoric acid) (10:90, v/v), which was supplemented with exactly 3 mL of the internal standard solution (0.4 mg/mL brucine dihydrate in the mobile phase) and with the mobile phase to produce 25 mL of sample solutions.

Aralia rhizome: The *Aralia* rhizome powders (500 mg) were precisely weighed and extracted with 30 mL of methanol under reflux conditions for 30 min, then cooled and filtered. The residues were supplemented with 30 mL of methanol and processed as before with two repetitions. After extraction, all the filtrates of each sample were combined and supplemented with methanol to produce exactly 100 mL of sample solutions.

Anemarrhena rhizome: The *Anemarrhena* rhizome powders (500 mg) were precisely weighed and extracted with 30 mL of 90% methanol (v/v) under reflux conditions for 30 min, then cooled and filtered. The residues were supplemented with 30 mL of 90% methanol (v/v) and processed as before with two repetitions. After extraction, all the filtrates of each sample were combined and supplemented with 90% methanol (v/v) to produce exactly 100 mL of sample solutions.

All the samples were filtered through the 0.45-μm PTFE membrane and stored at 4°C for further analysis.

(7) Assay of Active Ingredients via LC-MS Analysis

LC-MS analyses were performed using a Shimadzu LCMS-IT-TOF Mass Spectrometer (Shimadzu, Kyoto, Japan) equipped with an ESI interface. ESI parameters were as follows: source voltage +4.5 kV; capillary temperature 200°C; and nebulizer gas 1.5 L/min. The mass spectrometer was operated in the positive and negative ion scanning modes from 150 to 1500 *m/z*. A Waters Atlantis T3 column (2.1 mm × 150 mm, 5 μm) was used at a column temperature maintained at 40°C. The mobile phase was a binary eluent of (A) 5 mM CH_3COONH_4 solution and (B) CH_3CN under the following gradient conditions: 0–30 min linear gradient from 10% to 100% B, 30–40 min isocratic at 100% B. The flow rate was 0.2 mL/min.

(8) High Performance Liquid Chromatography (HPLC) Analysis

The HPLC system used was a Shimadzu LC-20AT system (Shimadzu Corporation, Kyoto, Japan) equipped with a Shimadzu SPD-20A detector. A Cosmosil $5C_{18}$-MSII column (4.6 mm × 150 mm, 5 μm; Nacalai Tesque, Inc., Kyoto, Japan) was used. The analytical conditions were prepared

according to the Japanese Pharmacopoeia, 18th Edition (Ministry of Health, Labour and Welfare 2021) as follows.

Scutellaria root: The mobile phase was acetonitrile and 0.68% phosphoric acid solution (7:18, v/v), the flow rate was 0.75 mL/min, and the detection wavelength was 277 nm.

Peony root: The mobile phase was acetonitrile mixed with phosphoric acid solution and water (150:1:850, by vol), the flow rate was 0.8 mL/min, and the detection wavelength was 232 nm.

Scopolia rhizome: The mobile phase was acetonitrile and 0.05 mol/L potassium dihydrogen phosphate solution (containing 1% triethylamine, adjusted to pH 3.5 with phosphoric acid) (10:90, v/v), the flow rate was 1.0 mL/min, and the detection wavelength was 210 nm.

Aralia rhizome: The mobile phase was acetonitrile and 0.1% trifluoroacetic acid solution (99:1, v/v), the flow rate was 0.5 mL/min, and the detection wavelength was 205 nm.

(9) Statistical Analysis

All data obtained were parametric and expressed as mean ± standard deviation. The results were analyzed by one-way analysis of variance using SPSS Statistics version 25. Statistical differences were considered significant at $p < 0.05$.

3. Results and Discussion

(1) Effect of Microbubble Washing on Microbial Load

We removed the initially adhered sediment cleanly after microbubble washing (Figure 1). Therefore, we tested for residual microorganisms.

The effects of the three processing methods on the total bacterial count (TBC) and coliform count (CFC) of the five crude drugs are shown in Table 1 and Table 2. After the microbubble processing, the total bacterial counts of the two crude drugs (i.e., *Scopolia* rhizome and peony root) significantly decreased; *Scopolia* rhizome, 101.0 ± 17.7–19.0 ± 9.6 CFU/mL; and peony root, 31.5 ± 4.9–4.7 ± 3.1 CFU/mL. However, the bactericidal effects of *Scutellaria* root, *Aralia* rhizome, and *Anemarrhena* rhizome were not sufficient (*Scutellaria* root, 116.3 ± 58.8–105.7 ± 62.7 CFU/mL; *Aralia* rhizome, 59.3 ± 42.0–20.3 ± 15.5 CFU/mL; *Anemarrhena* rhizome, 695.3 ± 164.0–604.3 ± 151.6 CFU/mL). Concerning the coliform counts, except for *Scutellaria* root and *Anemarrhena* rhizome, we observed bactericidal effects in the case of three crude drugs (*Scopolia* rhizome, 19.0 ± 8.5–2.3 ± 1.5 CFU/mL; *Aralia* rhizome, 6.0 ± 6.0–0.7 ± 1.2 CFU/mL; peony root, 0.3 ± 0.6–0 CFU/mL).

In the case of certain crude drugs, microbubble washing was sufficient for sterilization. However, some crude drugs display vertical wrinkles and structural features that hinder cleaning, such as *Scutellaria* root or *Anemarrhena* rhizome, respectively (Figure 1), and cannot be sufficiently sterilized by microbubble washing alone. Sodium hypochlorite (NaClO) is a disinfectant commonly used in the food industry to eliminate microorganisms (Francisco et al. 2018; Fukuzaki 2006; Ushida et al. 2017). Therefore, sodium hypochlorite was added to the microbubbles to enhance the cleaning effect.

The addition of sodium hypochlorite to the microbubbles markedly reduced the colony number, even for those with vertical wrinkles, such as *Scutellaria* root (Tables 1 and 2). Nevertheless, even microbubbles with sodium hypochlorite did not produce sufficient bactericidal effects in crude drugs with leaf-derived hair-like structures, such as *Anemarrhena* rhizome (Figure 1). Zhang et al. have reported that surface roughness and hydrophobicity affect the bactericidal effect

of microbubble cleaning (Zhang and Tikekar 2021). Generally, the surfaces of aerial parts of plants are hydrophobic. Thus, in the case of *Anemarrhena* rhizomes, it is considered that the remaining hydrophobic leaf-derived hair-like structures prevent adequate cleaning. In addition, a combination of microbubbles and water circulation is necessary to achieve sufficient bactericidal effect. However, water circulation is difficult to achieve in a confined space. Therefore, water circulation did not occur inside the vertical wrinkles of *Scutellaria* root, and sufficient bactericidal effect could not be obtained. In such cases, longer washing times were required. In the previous study (Ushida et al. 2017), Chinese cabbage was washed with alternating flow microbubbles for 3 mins, but it did not significantly reduce viable bacteria count, also suggesting longer washing is needed. Klintham et al. (Klintham et al. 2017) found that 5 mins of microbubble washing reduced coliform colonies on vegetables, with NaClO addition enhancing sterilization, which is consistent with our findings. In addition, according to Food and Drug Administration regulations, the allowable concentration range of NaClO for use as a food disinfectant is 50–200 ppm (0.005%–0.02%). Thus, disinfection can also be implemented at higher concentrations than that used in this experiment (Oliveira et al. 2012).

Table 1. Effects of Three Disinfection Processes on the Total Bacterial Count (TBC) of Five Crude Drugs.
Values were presented as the mean ± standard deviation (SD) of triplicate experiments.

Crude drugs	TBC (CFU/mL)		
	Control	Microbubble	Microbubble + NaClO
Scopolia rhizome	101.0 ± 17.7	19.0 ± 9.6	4.3 ± 2.1
Scutellaria root	116.3 ± 58.8	105.7 ± 62.7	35.3 ± 7.0
Aralia rhizome	59.3 ± 42.0	20.3 ± 15.5	12.0 ± 8.0
Anemarrhena rhizome	695.3 ± 164.0	604.3 ± 151.6	373.3 ± 148.6
Peony root	31.5 ± 4.9[#]	4.7 ± 3.1	1.3 ± 1.5

#Calculated by two data sets due to the subsistence of extreme data.

Table 2. Effects of Three Disinfection Processes on the Coliform Count (CFC) of Five Crude Drugs.
Values were presented as the mean ± standard deviation (SD) of triplicate experiments.

Crude drugs	CFC (CFU/mL)		
	Control	Microbubble	Microbubble + NaClO
Scopolia rhizome	19.0 ± 8.5	2.3 ± 1.5	1.0 ± 1.0
Scutellaria root	4.7 ± 6.4	1.3 ± 1.5	0
Aralia rhizome	6.0 ± 6.0	0.7 ± 1.2	0.3 ± 0.6
Anemarrhena rhizome	14.7 ± 11.0	11.7 ± 8.1	10.0 ± 8.9
Peony root	0.3 ± 0.6	0	0

Figure 1. Pictures of Crude Drugs.
(1) *Scopolia* rhizome, (2) *Scutellaria* root, (3) *Aralia* rhizome, (4) *Anemarrhena* rhizome, (5) Peony root.

(2) Effect of Microbubble Treatment on the Active Ingredient Concentrations in Crude Drugs

Avoiding active ingredient elution is important in the current crude drug washing process. Therefore, we examined the content-related changes of the main components of each crude drug upon microbubble washing.

Each of the five crude drugs was analyzed for a specific active ingredient, as follows: *Scopolia* rhizome, alkaloids; *Anemarrhena* rhizome, saponins; *Aralia* rhizome, diterpenes; peony root, monoterpene glycosides; and Scutellaria root, flavonoids. The evaluation of the index compounds was in accordance with guidelines in the Japanese Pharmacopoeia (Ministry of Health, Labour and Welfare 2021). The total ion chromatograms (TIC) of the extracts from the five crude drugs and standards are shown in Figures 2–4. The results of HPLC and LC-MS indicated no significant differences in the concentrations of the main active ingredients among control, microbubble wash, and microbubble + 0.01% NaClO wash ($p > 0.05$) (Table 3). No significant changes in peak intensity were observed in the TIC for the other components of the five crude drugs evaluated in this study (See figures 2-4 after Section 4).

Table 3. Major Index Compounds Found in Five Crude Drugs.
Values were presented as the mean ± standard deviation (SD) of triplicate experiments. The statistical differences were significant at $p < 0.05$.

Crude drugs	Compounds	Active ingredients content (%)			
		Control	Microbubble	Microbubble + NaClO	*p*-value
Scopolia rhizome	Atropine	0.12 ± 0.07	0.10 ± 0.04	0.13 ± 0.06	0.8002
	Scopolamine	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	0.6111
Scutellaria root	Baicalin	16.6 ± 1.3	19.4 ± 2.0	17.9 ± 1.5	0.1985
Aralia rhizome	Kaurenoic acid	0.94 ± 0.47	0.87 ± 0.40	0.90 ± 0.46	0.9817
Anemarrhena rhizome	Anemarsaponin B	0.17 ± 0.04	0.26 ± 0.10	0.16 ± 0.04	0.2366
	Timosaponin AIII	0.28 ± 0.16	0.24 ± 0.10	0.28 ± 0.02	0.8944
Peony root	Paeoniflorin	4.1 ± 1.0	4.5 ± 1.3	4.4 ± 1.4	0.8947

4. Conclusion

Our data demonstrated that microbubble washing could reduce the number of viable bacteria and coliforms in the case of *Scopolia* rhizome, *Aralia* rhizome, and peony root, with a notable

ability to eradicate bacteria. However, we could not achieve sufficient sterilization in the case of *Scutellaria* root and *Anemarrhena* rhizome, potentially due to the structural features of these samples; coarse and marked longitudinal wrinkles with scattered scars of the lateral root and brown periderm remain on the *Scutellaria* root (Figure 1), or rather, flat and cord-like rhizomes with longitudinal furrows and hair-like remains or scars of leaf sheaths forming fine ring-nodes (on the upper surface) or scars of roots appearing as numerous round spot-like hollows (on the lower surface) on the *Anemarrhena* rhizome (Figure 1). However, as an increase in cleaning time would cause the risk of active ingredient elution, we supplemented the microbubbles with sodium hypochlorite (NaClO), a commonly used disinfectant in the food industry, to further increase the sterilization effect, and we observed a significantly improved sterilization rate. As our analyses have revealed, microbubble washing does not significantly affect the concentrations of the main bioactive phytochemicals in the five crude drugs, even with the addition of NaClO. These results indicate that compared to simple water rinsing, microbubble rinsing is an effective method to simultaneously clean and sterilize crude drugs, and at the same time, provides the advantage of shorter rinsing time and less water resource waste. In addition, the sterilizing effect could be further enhanced by NaClO supplementation.

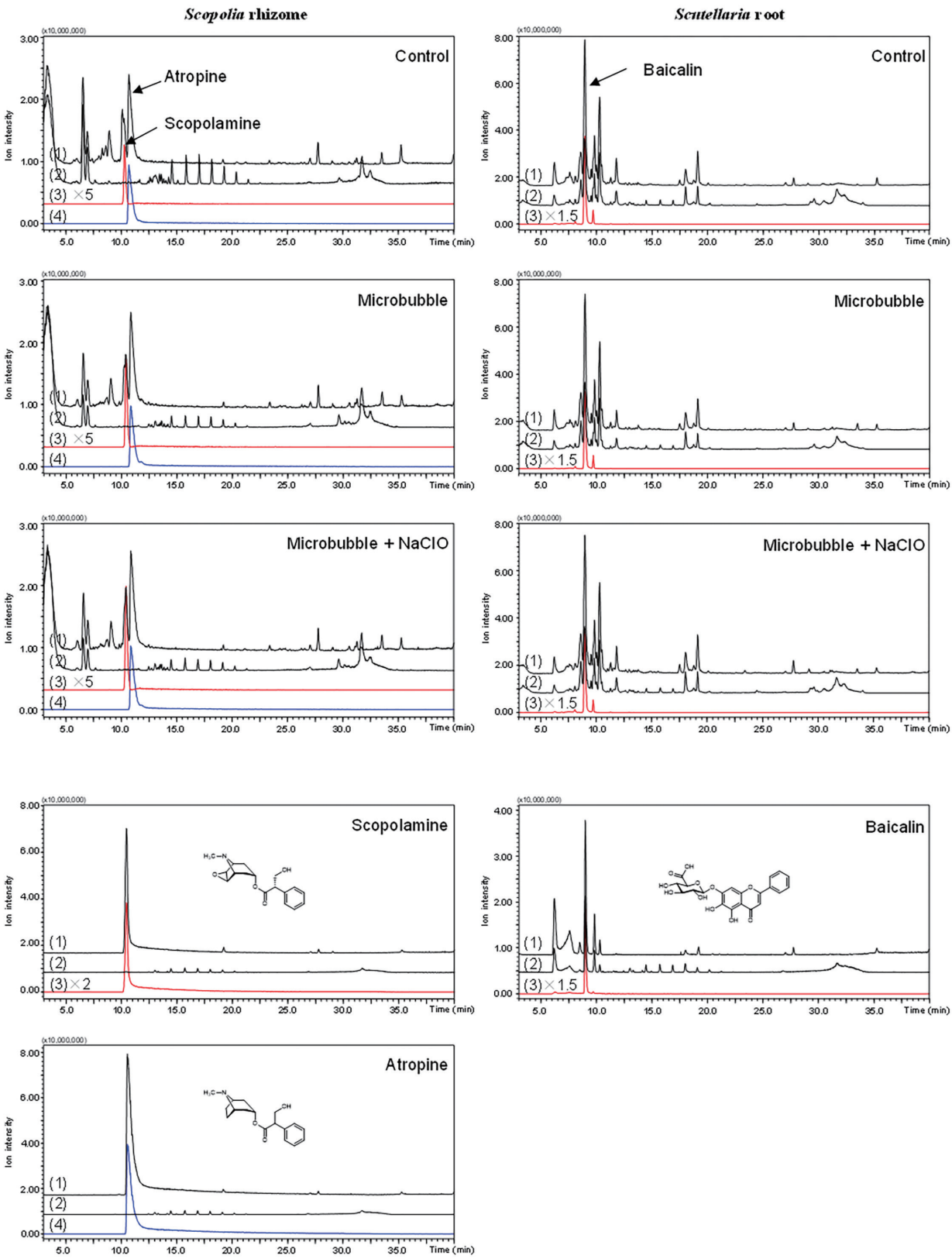

Figure 2. Total Ion Chromatograms (TIC) of the *Scopolia* Rhizome and *Scutellaria* Root Extracts. *Scopolia* rhizome: (1) TIC in positive ion mode, (2) TIC in negative ion mode, (3) *m/z* 304.1546 ($[M + H]^+$ of scopolamine), (4) *m/z* 290.1760 ($[M + H]^+$ of atropine). *Scutellaria* root: (1) TIC in positive ion mode, (2) TIC in negative ion mode, (3) *m/z* 447.0956 ($[M + H]^+$ of baicalin).

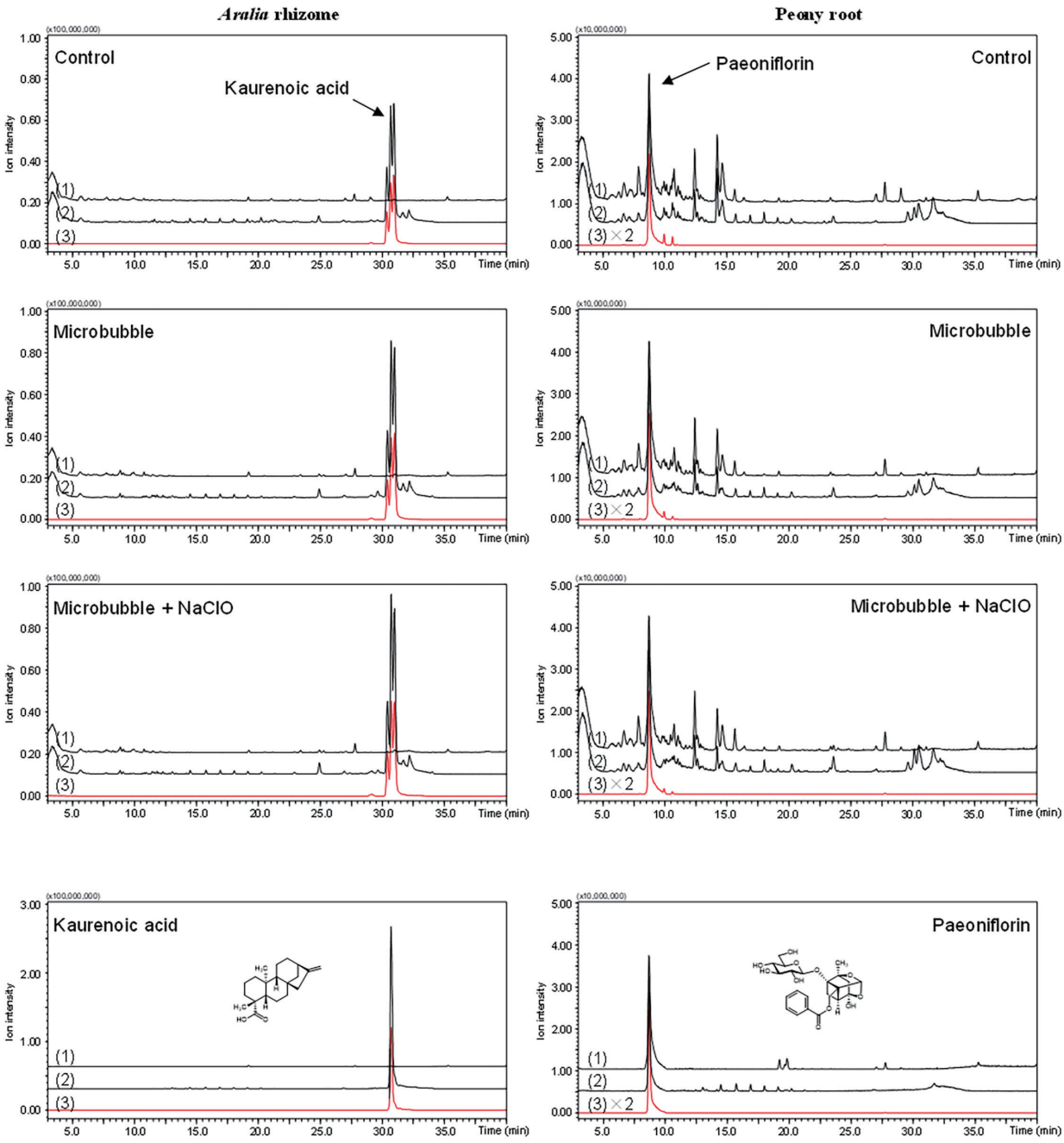

Figure 3 Total Ion Chromatograms (TIC) of the *Aralia* Rhizome and Peony Root Extracts. *Aralia* rhizome: (1) TIC in positive ion mode, (2) TIC in negative ion mode, (3) *m/z* 301.2174 ($[M - H]^-$ of kaurenoic acid). Peony root: (1) TIC in positive ion mode, (2) TIC in negative ion mode, (3) *m/z* 498.1998 ($[M + NH_4]^+$ of paeoniflorin).

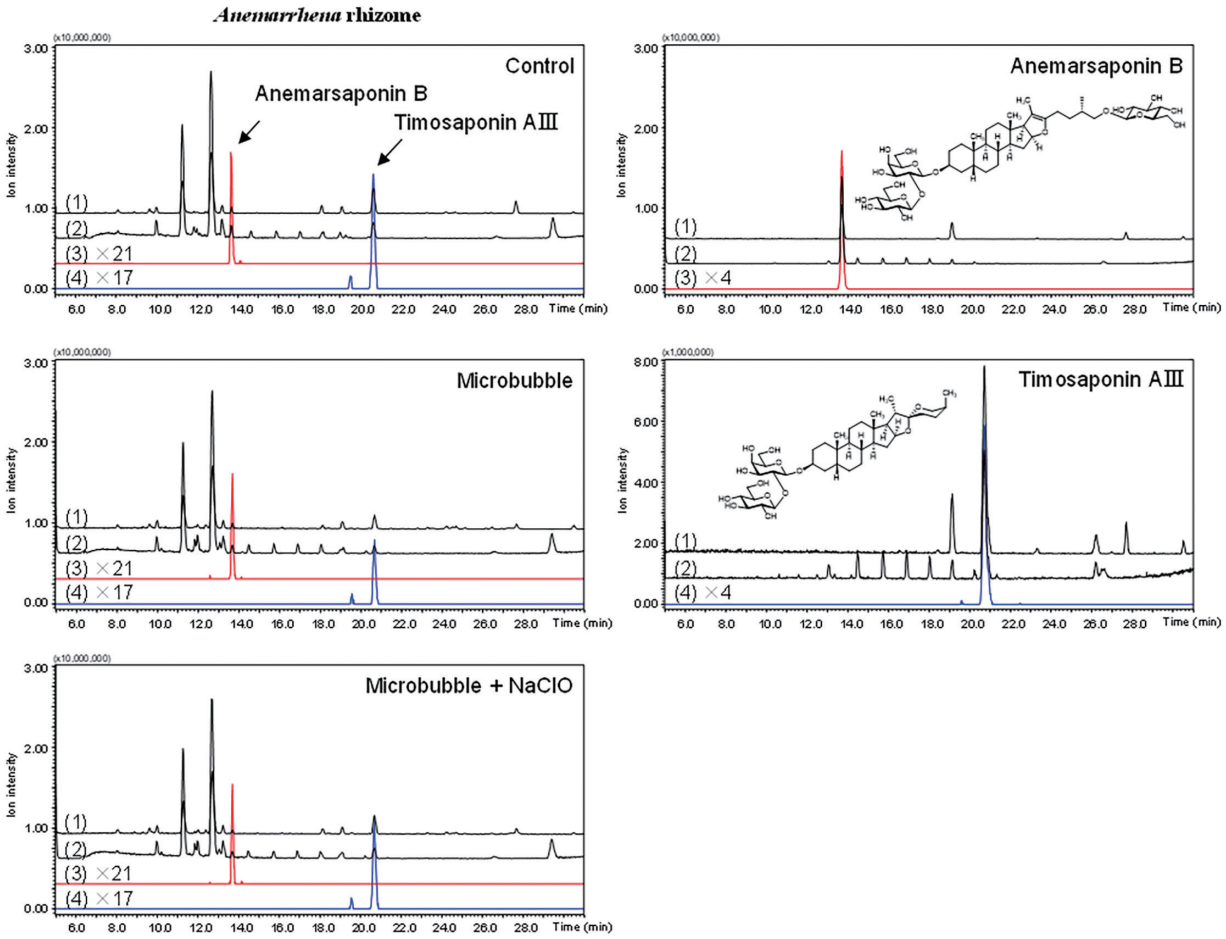

Figure 4. Total Ion Chromatograms (TIC) of the Extracts from *Anemarrhena* Rhizome.
Anemarrhena rhizome: (1) TIC in positive ion mode, (2) TIC in negative ion mode, (3) *m/z* 480.2423 ($[M + CH_3COO - H]^{2-}$ of anemarsaponin B), (4) *m/z* 739.4268 ($[M - H]^-$ of timosaponin AIII).

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