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Cinnamon (*Cinnamomum cassia*) water extract improves diarrhea symptoms by changing the gut environment: a randomized controlled trial†

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Cinnamon is a spice obtained from the bark of *Cinnamomum* and contains anti-inflammatory ingredients such as coumarin, cinnamaldehyde, and cinnamic acid. This study evaluated the effect of cinnamon water extract (CWE) on the symptoms of subjects with diarrhea in an 8-week randomized controlled trial. Seventy subjects with diarrhea symptoms were randomized and received three capsules of 400 mg CWE or placebo twice daily for 8 weeks. CWE intake significantly increased colonic transit time (p = 0.019) and fecal isobutyric acid (p = 0.008) and spermidine (p = 0.009) contents compared to placebo intake. In contrast, CWE decreased fecal indole (p = 0.032) and agmatine (p = 0.018) contents. Gut microbiota analysis showed increased alpha diversity and significant changes in strains such as *Bifidobacterium longum ATCC 55813* (LDA = 1.38) in the CWE group compared with the placebo group. *Bifidobacterium longum ATCC 55813* showed a positive correlation with colon transit time and stool phenol and spermidine contents. CWE improved diarrhea symptoms and changed the composition of stools and the gut microbiota. These results indicate that cinnamon intake relieves diarrhea symptoms through metabolic changes due to changes in intestinal microbial groups.

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Introduction

Diarrhea is a common condition that occurs through disruptions in gut immune function and homeostasis. Chronic diarrhea is a frequent symptom for both general physicians and gastroenterologists. The differential diagnosis is extensive and can be complex in diagnostic evaluation.[1,2] Chronic diarrhea, especially functional diarrhea, is a significant clinical problem. Despite a large number of causes of chronic diarrhea and extensive investigations, no cause of symptoms was found in a large number of patients with functional diarrhea.[3] According to the Rome III criteria for diagnosing functional gastrointestinal disorders, functional diarrhea is defined as having loose or watery feces in at least 75% of the feces in the absence of pain.[4] There is growing evidence that an imbalance in the intestinal microbiota increases susceptibility to various pathogens and causes many diseases, such as diarrhea, irritable bowel syndrome (IBS), allergies, cardiovascular diseases, and obesity.[5]

Food digested in our bodies goes to the gut and is metabolized by the gut microbiota.[6] The gut microbiota preferentially ferments carbohydrates from nonprotein energy sources, and it can decrease indole production by tryptophan metabolism.[7] Microbial fermentation can contribute to the formation of toxic materials, such as *p*-cresol, which can cause cytotoxicity and reduce barrier protection.[8] Dietary fibers and carbohydrates enter the intestinal system and are fermented by microorganisms to form short-chain fatty acids (SCFAs).[9] SCFAs maintain intestinal mucosa homeostasis and contribute to mucus production capacity, immune function, and intestinal integrity.[10] In addition, biogenic amines produced by the microbiota act as important signaling factors between hosts and symbiotic microorganisms.[11]

Cinnamon is a spice obtained from the bark of *Cinnamomum* and has been widely used for medicinal purposes. *Cinnamomum cassia* bark has treatment effects on inflammation.[12] In addition, bioactive materials in *C. cassia*, such as coumarin, cinnamaldehyde, and cinnamic acid, can reduce the inflammatory response by inhibiting nuclear factor-

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† Electronic supplementary information (ESI) available: Table S1: Baseline demographic characteristics of study participants. Table S2: Compliance. Table S3: Dietary intake and physical activity. Table S4: Vital signs. Table S5: Hematological test results. Table S6: Blood chemistry test results. Table S7: Urine test results. Table S8: Normal ranges for hematological test, blood chemical test, and urine test results. Fig. S1: Altered microbiota composition after the ingestion of CWE, analyzed at the (A) family and (B) genus level. See DOI: https://doi.org/10.1039/d2fo01835g

κB-associated pathways.[13,14] Cinnamon is also known to have an inhibitory effect on the growth of certain microbes *in vitro*.[15] However, the effect of *C. cassia* water extract on intestinal health has not been elucidated through clinical trials.

In this study, it was hypothesized that cinnamon water extract (CWE) prepared through the hot water extraction method would improve diarrhea symptoms in a clinical model by altering the intestinal environment and microbial fermentation products. The relationship between the analyzed biomarkers and gut microbiota was determined through correlation analysis.

Materials and methods

Study materials

CWE was manufactured by Dainnatural (Cheonan, Korea). The *C. cassia* branch was extracted with hot water as a solvent. Briefly, 100 kg of *C. cassia* branch were mixed with 10 times distilled water (w/v), and extraction was performed at 121 °C for 8 h. The specimens were filtered and evaporated. After evaporation under reduced pressure at a low temperature, the concentrated extract was converted into dried particles by spray drying.

The experimental capsule was formulated as 96.85% cinnamon extract, 0.97% magnesium stearate, 1.94% silicon dioxide, and 0.24% flavoring. The placebo capsule was formulated as 92.58% corn starch, 0.97% magnesium stearate, 6.22% pigments, and 0.23% flavoring. The extract was formulated to contain 400 mg CWE per capsule. Samples were stored below refrigeration temperature, avoiding sunlight. Subjects received three capsules of CWE or placebo twice daily for 8 weeks. Consumption of 2400 mg of CWE was determined from our previous study[16] and calculated as follows. As a result of our previous study, the effect of 500 mg kg^{-1} cinnamon water extract on gut health improvement was confirmed using a mouse colitis model induced by 5% dextran sodium sulfate.

$$\text{Human equivalent dosage(HED)} = \text{animal dose}(\text{mg kg}^{-1}) \frac{\text{animal } Km}{\text{human } Km}$$

$$^{*}Km = \text{body weight(kg)} \div \text{surface area}(\text{m}^2)$$

$$(\text{mouse}, 3;\ \text{human}, 37)$$

Quantification of bioactive compounds by ultrahigh-performance liquid chromatography (UHPLC)

For the detection of the compounds in CWE, quantitative analysis was performed on a Vanquish UHPLC coupled with a TSQ Altis (Thermo Fisher, Waltham, MA, USA). Analytes were separated on a Waters Cortects C_{18} (1.6 μm, 150 mm × 2.1 mm) column by gradient elution using mobile phase A (0.1% formic acid in D. W) and mobile phase B (0.1% formic acid in acetonitrile) at 0.25 mL min^{-1}. Heated electrospray ionization (H-ESI) parameters were as follows: positive ion spray voltage: 3500 V, negative ion spray voltage: 2500 V; sheath gas: 50 (arbitrary units); auxiliary gas: 10 (arbitrary units); sweep gas: 1 (arbitrary units); ion transfer tube temperature 325 °C; vaporizer temperature 350 °C. The triple quadrupole was operated in the selected reaction monitoring (SRM) mode. The samples were determined in triplicate.

Subjects and study design

The study protocol was approved by the Institutional Review Board and Ethics Committees of the Jecheon Oriental Medicine Hospital affiliated with Semyung University (Jecheon, Korea) (approval number 2018-02-02), where the research subjects were recruited, and the Clinical Research Information Service (registration number KCT0003399). Biomarker analysis was performed at Seoul National University of Science and Technology. The study was designed as a randomized, double-blind, parallel, and placebo-controlled study. Randomization was accomplished with a computer-generated random list. Block randomization designs were used to balance the sample size across groups. In this study, sample size determination was performed as follows, based on the comparison means between the two groups. If the observations came from a normal distribution, then with the anticipated standardized effect size between them, based on the results of Shin *et al.*'s study,[17] specified as a result of assuming that the mean ± SD for the change in the number of bowel movements is −0.50 ± 0.64 times, the sample size in each group for a two-sided test $\alpha = 0.05$ and power $1 - \beta = 0.8$ was 52. Therefore, the number of target subjects per group was 26 each, and a total of 70 subjects were registered (35 per group), considering the 25% dropout rate.

The inclusion criteria were as follows: adults over 20 years of age with diarrhea according to the Rome III criteria (functional diarrhea). The exclusion criteria were as follows: consuming products that affect intestinal function for more than a week within 4 weeks of the first visit; having irritable bowel syndrome, alcoholism, hypertension, cardiovascular disease, immune disease, mental disease, liver function disorder, or renal dysfunction; having high dietary fiber intake; having hypersensitivity to the ingredients within the test food; participating in another clinical study within 4 weeks of the first visit; being pregnant or lactating; and being judged by the researcher as ineligible to participate in this study. Written informed consent was obtained from all subjects prior to study initiation.

After a run-in period of 1 week, subjects were randomly assigned to the placebo group or the test group according to the order registered at visit 2 (week 0). Food containing cinnamon, probiotics, and prebiotics, as well as products in the form of pills and extracts and herbal medicines and drugs, were prohibited until the end of the study. Fruit intake was limited to 2 servings per day, and vegetable intake was limited to 6 servings per day. To compare the differences in nutrient intake among subjects, the dietary intake contents for 3 days (2 weekdays, 1 weekend), representative of the usual diet, were submitted with a smartphone application or diary prior to the study visit. Except for the intake standards, subjects were instructed to maintain a usual diet and lifestyle. To ensure

safety, vital sign examinations and clinical pathology and urine tests were performed, and adverse reactions were recorded. Compliance was checked by counting the remaining sample capsules. At 0 and 8 weeks from the start of the test, 10 mL of venous blood, 15 mL of urine, and stool samples were collected.

Assessment of bowel habits

To investigate the effects of CWE on bowel habits, the CTT after sample intake was measured using radio-opaque Kolomark markers (MI Tech, Pyeongtaek, Korea). After 72 hours and 144 hours, abdominal images were taken in the supine position, including the diaphragm and pubis. The CTT value was defined as multiplied by 1.2 by adding all the remaining markers as a result of the imaging. The percentage of bowel movements with diarrhea, satisfaction with defecation, and Bristol stool scale score were recorded as weekly averages. Abdominal pain, urgency, and distension were surveyed at each visit using a visual analog scale (VAS).

Assessment of fecal contents

Phenolic compounds and SCFAs were analyzed with a GC Agilent 6890 N system (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. The compounds were analyzed by the method described in Flickinger *et al.*[18] and Park *et al.*[19] with a DB-17 column (0.25 μm, 30 m × 0.25 mm; Agilent Technologies). 4-Isopropylphenol (Sigma Aldrich Corporation. Ltd, St Louis, MO, USA) was used as an internal standard. The fecal samples were dissolved in 1.0 mL of acetonitrile, vortexed, and sonicated. The emulsion was centrifuged at 3000 rpm for 5 min at 4 °C, and the supernatants were used for analysis.

To analyze SCFAs in feces, the method of Costabile *et al.* was used.[20] Branched SCFAs were used as external standards (Sigma Aldrich Corporation). 2-Ethyl butyric acid was used as an internal standard. Analysis was performed using a DB-FATWAX UI column (0.25 μm, 30 m × 0.25 mm; Agilent Technologies).

Biogenic amines in feces were analyzed with a Shiseido SI-2 HPLC system with a fluorescence detector (Shiseido, Kyoto, Japan) using Saarinen's method.[21] The separation of amine components was performed with a Cadenza 5CD-C18 (5 μm, 250 mm × 3 mm) column equipped with a Cadenza 5CD-C18 Guard Cartridge (5 μm, 5 mm × 2 mm; Imtakt USA, Portland, OR, USA). Using heptylamine as an internal standard, the total amounts of each component were summed to obtain the total amine concentration.

Microbiota analysis

The analysis was performed on stool samples obtained before and after CWE ingestion from 16 subjects randomly selected from the CWE group. Stool samples were diluted 1 : 10 in 10 mL of sterilized PBS for 24 hours and filtered with a cell strainer (SPL Life Sciences Co., Ltd, Pocheon, Republic of Korea). Bacteria in stools were isolated by centrifugation at 10 000*g* for 10 min at 4 °C. The pellet was boiled for 40 min at 100 °C, and a DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) was used to extract bacterial DNA. Bacterial DNA was quantified by using a QIAxpert system (QIAGEN), and the V3–V4 regions of 16S rDNA were amplified with a MiSeq system (Illumina, San Diego, USA). Taxonomy assignment was carried out by using UCLUST and QIIME against the Greengenes database.

Alpha diversity was assessed using the ACE, Chao1, Observed, Shannon, Simpson, and Fisher indices to compare gut microbiota diversity. Linear discriminant analysis of effect size (LEfSe) analysis was performed to confirm alterations in microbes due to CWE treatment.[22] A logarithmic linear discriminant analysis (LDA) score of more than one point was considered to represent a significant difference between two different groups. Correlations with functional markers present in feces were analyzed using Spearman's method for species showing differences before and after CWE ingestion.

Statistical analysis

The analysis was performed using the intention-to-treat method. For comparisons of subject characteristics between groups at baseline, Student's *t* test was performed for continuous variables. The chi-square test or Fisher's exact test was performed for categorical variables. Intergroup comparisons of compliance were analyzed by Student's *t* test. To analyze the differences between groups according to the intake period and for group comparisons before and after the intake period, evaluation variables were calculated from a linear mixed-effect model in consideration of group, time (week), and the group × time interaction (group × week). Correlations between biomarkers were analyzed using Spearman's method. Statistical analysis was performed using Statistical Analysis System (Version 9.4; SAS Institute, Cary, NC, USA). Statistical significance was set at $p < 0.05$.

Results

Baseline characteristics of subjects

The Consolidated Standards of Reporting Trials (CONSORT) flow diagram of this study is shown in Fig. 1. A total of 76 subjects were screened, and 70 subjects who satisfied the inclusion and exclusion criteria were enrolled in the study. The subjects were randomly assigned to either the placebo group or the CWE group, with 35 subjects in each group. Sixty-seven subjects completed the experiment (placebo group, 33 subjects; CWE group, 34 subjects). The analysis was performed with the intention-to-treat method. There were no significant differences in the baseline characteristics (Table S1†) or compliance at the endpoint between groups (Table S2†). The baseline clinical features of the subjects were within the normal range (Tables S3–8†). There were no reports of adverse reactions related to the test materials, and there were no significant adverse events related to the test materials.

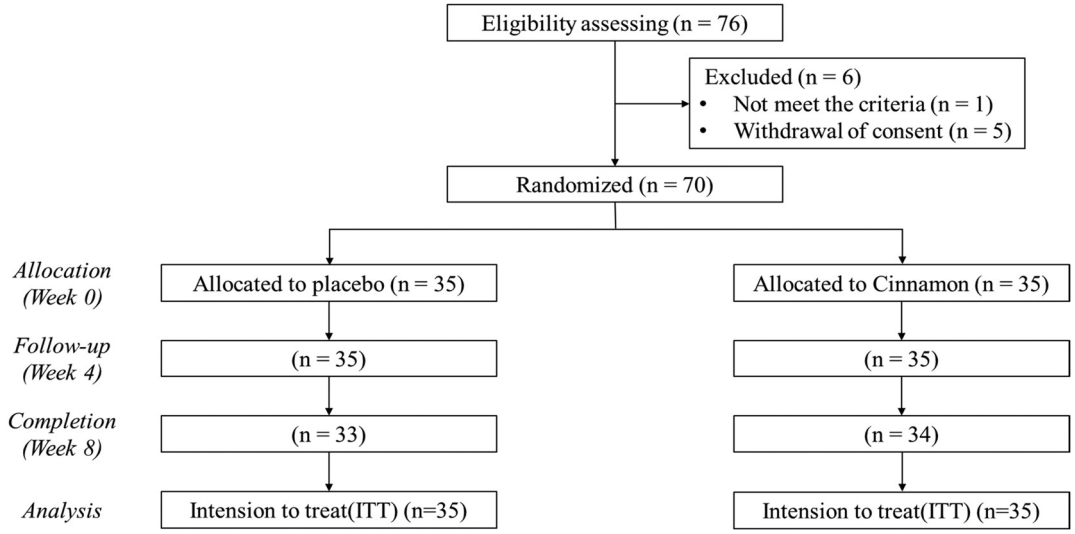

Fig. 1 CONSORT flow diagram of the study. Two people from the placebo group and one person from the CWE group dropped out. A total of 67 people completed the experiment.

Bioactive compounds in CWE

Several bioactive components of the CWE are shown in Table 1. Chlorogenic acid, cinnamaldehyde, cinnamic acid, coumarin, isovanillin, *p*-coumaric acid, protocatechuic acid, quercitrin, salicylic acid, and sinapyl aldehyde were identified and quantified. The highest values were observed in coumarin (9805.88 ± 95.57 mg kg^{-1}), followed by cinnamic acid (2920.83 ± 18.96 mg kg^{-1}).

CWE improved CTT

Bowel habits were investigated to determine whether diarrhea symptoms were improved. CWE intake prevented a decrease in CTT, while CTT decreased from 25.6 ± 3.4 hours to 15.6 ± 3.4 hours in the placebo group ($p < 0.05$) and showed a significant difference in the group × week interaction ($p = 0.019$) (Table 2). Subjects with a percentage of bowel movements with diarrhea of 75% or higher who consumed CWE showed improvements in VAS scores, the percentage of bowel movements with diarrhea, satisfaction with defecation, and Bristol stool scale scores, but these improvements were not statistically significant compared to the placebo group.

Table 1 The concentration of bioactive compounds of CWE[a]

Compounds	Concentration (mg kg^{-1})
Chlorogenic acid	100.65 ± 1.35
Cinnamaldehyde	15.33 ± 0.23
Cinnamic acid	2920.83 ± 18.96
Coumarin	9805.88 ± 95.57
Isovanillin	87.53 ± 0.64
p-Coumaric acid	168.22 ± 1.98
Protocatechuic acid	1138.44 ± 42.63
Quercitrin	29.17 ± 0.54
Salicylic acid	119.66 ± 0.83
Sinapyl aldehyde	131.95 ± 0.62

[a] Data are described as the mean ± standard deviation.

CWE changed fecal contents

The contents of indole, phenol, SCFAs, and biogenic amines in the feces changed with the consumption of CWE. No significant change was found in the indole content of the placebo group; however, the indole content decreased significantly, by 21.8%, from 21.6 ± 2.0 to 16.9 ± 2.0 mg g^{-1} in the CWE group ($p < 0.05$), demonstrating a significant group × week interaction ($p = 0.032$). Except for skatole, other phenolic compounds decreased in the placebo group and increased in the CWE group, but there was no significant difference. CWE did not change the stool skatole content, *p*-cresol content, pH, or water content (Table 2).

As a result of analyzing the content of SCFAs in stool samples, no significant change was found in the fecal isobutyric acid content with placebo ingestion; conversely, CWE ingestion significantly increased the isobutyric acid content, from 0.561 ± 0.011 to 0.573 ± 0.011 mg g^{-1} ($p < 0.05$), and it showed a significant difference in the group × week interaction ($p = 0.008$). CWE did not change the content of other SCFAs (Table 2).

The fecal biogenic amine contents of the subjects were confirmed. Agmatine in the placebo group showed no significant change, but CWE intake led to a 93.7% decrease in agmatine, from 429.4 ± 98.2 to 27.2 ± 105.6 μg g^{-1} ($p < 0.05$), and it demonstrated a significant difference in the group × week interaction ($p = 0.018$). Spermidine significantly decreased by 30.2%, from 471.7 ± 51.3 to 329.5 ± 53.6 μg g^{-1} ($p < 0.05$), in the placebo group, while the CWE group showed no significant difference. As a result, a significant difference in the group × week interaction was found for spermidine content ($p = 0.009$) (Table 2).

Table 2 Effect of cinnamon extract on the bowel habits of subjects (n = 70)[a]

Variables		Percentage of bowel movements with diarrhea	Placebo group (n = 35)		CWE group (n = 35)		p value[b]
			Week 0	Week 8	Week 0	Week 8	
Bowel habits	Colonic transit time (hours)	—	25.6 ± 3.4	15.6 ± 3.4	19.2 ± 3.4	21.7 ± 3.4	0.019*
	Percentage of bowel movements with diarrhea	75–100%	84.4 ± 11.5	51.8 ± 11.5	87.8 ± 18.8	22.9 ± 18.8	0.628
		50–74%	62.4 ± 12.0	28.4 ± 12.0	52.0 ± 12.0	36.0 ± 12.0	0.622
		25–49%	30.6 ± 7.6	0.8 ± 8.0	35.8 ± 9.3	28.9 ± 9.3	0.675
	Satisfaction with defecation (%)	75–100%	25.8 ± 13.6	48.4 ± 14.8	20.0 ± 22.2	58.1 ± 22.2	0.840
		50–74%	37.1 ± 13.3	75.4 ± 13.3	50.5 ± 13.3	66.0 ± 13.3	0.603
		25–49%	56.4 ± 11.5	58.2 ± 12.0	56.7 ± 14.0	48.9 ± 14.0	0.629
	Bristol stool scale score	75–100%	6.0 ± 0.2	5.5 ± 0.2	6.2 ± 0.3	5.9 ± 0.4	0.869
		50–74%	5.7 ± 0.3	5.4 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	0.645
		25–49%	5.8 ± 0.3	5.7 ± 0.5	6.0 ± 0.3	6.0 ± 0.3	0.885
	Abdominal pain	75–100%	44.2 ± 5.9	33.1 ± 6.1	36.5 ± 5.9	31.9 ± 5.9	0.730
		50–74%	48.3 ± 7.0	32.3 ± 7.4	41.3 ± 6.1	33.2 ± 6.3	0.615
		25–49%	35.9 ± 5.8	25.0 ± 5.8	23.5 ± 6.1	10.6 ± 6.1	0.886
	Bowel urgency	75–100%	42.3 ± 6.6	26.5 ± 6.8	45.4 ± 6.6	25.4 ± 6.6	0.368
		50–74%	61.7 ± 8.1	49.7 ± 8.4	44.2 ± 7.0	27.6 ± 7.2	0.422
		25–49%	36.5 ± 6.9	28.6 ± 6.9	21.5 ± 7.2	19.1 ± 7.2	0.868
	Abdominal distension	75–100%	43.1 ± 6.8	28.7 ± 7.0	49.2 ± 6.8	30.8 ± 6.8	0.907
		50–74%	43.3 ± 7.4	23.7 ± 7.8	45.4 ± 6.4	32.3 ± 6.6	0.819
		25–49%	37.0 ± 7.0	27.7 ± 7.0	34.5 ± 7.3	18.5 ± 7.3	0.768
Fecal condition	pH	—	6.4 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	0.380
	Water content (%)	—	79.6 ± 1.0	77.9 ± 1.0	79.2 ± 1.0	80.9 ± 1.1	0.107
Fecal indole and phenols (mg g^{-1})	Indole	—	20.3 ± 1.9	21.9 ± 1.9	21.6 ± 2.0	16.9 ± 2.0	0.032*
	Skatole	—	2.3 ± 0.8	3.8 ± 0.8	1.6 ± 0.8	2.4 ± 0.8	0.598
	Phenol	—	0.2 ± 0.7	0.7 ± 0.7	3.7 ± 0.7	3.1 ± 0.7	0.277
	p-Cresol	—	28.9 ± 4.4	31.4 ±.4.6	24.3 ± 4.7	22.9 ± 4.7	0.639
Fecal short-chain fatty acids (mg g^{-1})	Acetic acid	—	0.532 ± 0.009	0.526 ± 0.009	0.517 ± 0.009	0.524 ± 0.009	0.098
	Propionic acid	—	0.445 ± 0.006	0.442 ± 0.006	0.450 ± 0.006	0.446 ± 0.006	0.909
	Butyric acid	—	0.287 ± 0.001	0.286 ± 0.001	0.287 ± 0.001	0.286 ± 0.001	0.614
	Isobutyric acid	—	0.579 ± 0.011	0.569 ± 0.011	0.561 ± 0.011	0.573 ± 0.011	0.008*
	Valeric acid	—	0.389 ± 0.002	0.387 ± 0.002	0.389 ± 0.003	0.388 ± 0.003	0.394
	Isovaleric acid	—	0.493 ± 0.002	0.492 ± 0.002	0.499 ± 0.002	0.497 ± 0.002	0.434
Fecal biogenic amines (μg g^{-1})	Agmatine	—	217.6 ± 101.6	282.0 ± 98.1	429.4 ± 98.2	27.2 ± 105.6	0.018*
	Methylamine	—	1762.1 ± 251.2	1091.1 ± 261.6	1658.1 ± 258.9	910.5 ± 270.4	0.857
	Ethylamine	—	2966.1 ± 362.2	2910.3 ± 357.5	3895.3 ± 358.5	3363.3 ± 363.2	0.392
	Pyrrolidine	—	560.6 ± 140.5	661.8 ± 142.8	210.9 ± 153.3	452.3 ± 153.3	0.608
	Dimethylamine	—	127.2 ± 80.7	306.9 ± 77.5	300.0 ± 79.0	327.4 ± 80.0	0.217
	Propylamine	—	513.1 ± 62.4	460.4 ± 64.1	404.1 ± 63.0	390.7 ± 61.4	0.677
	Tryptamine	—	28.3 ± 5.9	12.6 ± 6.0	19.3 ± 5.8	2.7 ± 6.2	0.924
	Butylamine	—	2817.0 ± 460.4	2200.8 ± 472.1	3331.9 ± 455.0	1915.8 ± 492.2	0.255
	Phenylethylamine	—	129.9 ± 24.1	115.1 ± 25.6	95.6 ± 26.1	86.1 ± 26.1	0.909
	Putrescine	—	3591.2 ± 595.0	3225.8 ± 599.3	3919.3 ± 599.8	4447.6 ± 604.1	0.158
	Cadaverine	—	2557.2 ± 292.4	2096.6 ± 296.1	3345.1 ± 296.1	2787.8 ± 300.1	0.829
	Histamine	—	436.6 ± 71.6	324.7 ± 73.7	447.5 ± 70.5	370.4 ± 69.9	0.684
	Tyramine	—	3598.8 ± 754.2	3538.1 ± 764.1	3730.3 ± 754.2	3956.2 ± 756.4	0.568
	Spermidine	—	471.7 ± 51.3	329.5 ± 53.6	404.8 ± 52.1	501.4 ± 53.6	0.009*
	Spermine	—	1069.7 ± 116.6	1120.5 ± 120.6	911.3 ± 120.6	885.1 ± 116.6	0.727
	Total amine	—	27183.8 ± 2319.0	23136.3 ± 2338.3	27374.5 ± 2354.5	26 106.1 ± 2374.7	0.306

[a] Data were described as the LS mean ± standard error. [b] p values were calculated from a linear mixed-effect model to analyze the effect of the group × week interaction. *$p < 0.05$ indicates a statistically significant.

CWE altered the gut microbiota

The microbiota composition in stool samples was analyzed by 16S rRNA sequencing using feces obtained from the CWE group (n = 16). After CWE intake, *Verrucomicrobia*, *Cyanobacteria*, *Saccharibacteria*, and *Actinobacteria* were increased approximately 2.5-fold, 1.8-fold, 1.4-fold, and 1.2-fold, respectively, and at the phylum level, *Tenericutes* was reduced by one-quarter. Additionally, the Firmicutes/Bacteroidetes ratio decreased by 16%, confirming that Bacteroidetes increased relative to the phylum Firmicutes (Fig. 2A). Alpha-diversity analysis was performed to confirm changes in the richness and evenness of the intestinal microbiota (Fig. 2B). Significant differences were found in the observed, Chao1, ACE, Simpson, and Fisher indices but not in the Shannon index ($p < 0.05$).

LEfSe analysis was performed to confirm the change in c from the phylum to species level between the groups. In Fig. 2C, *Sellimonas* was significantly abundant before consuming CWE (LDA = −1.78). After CWE ingestion, *uncultured*

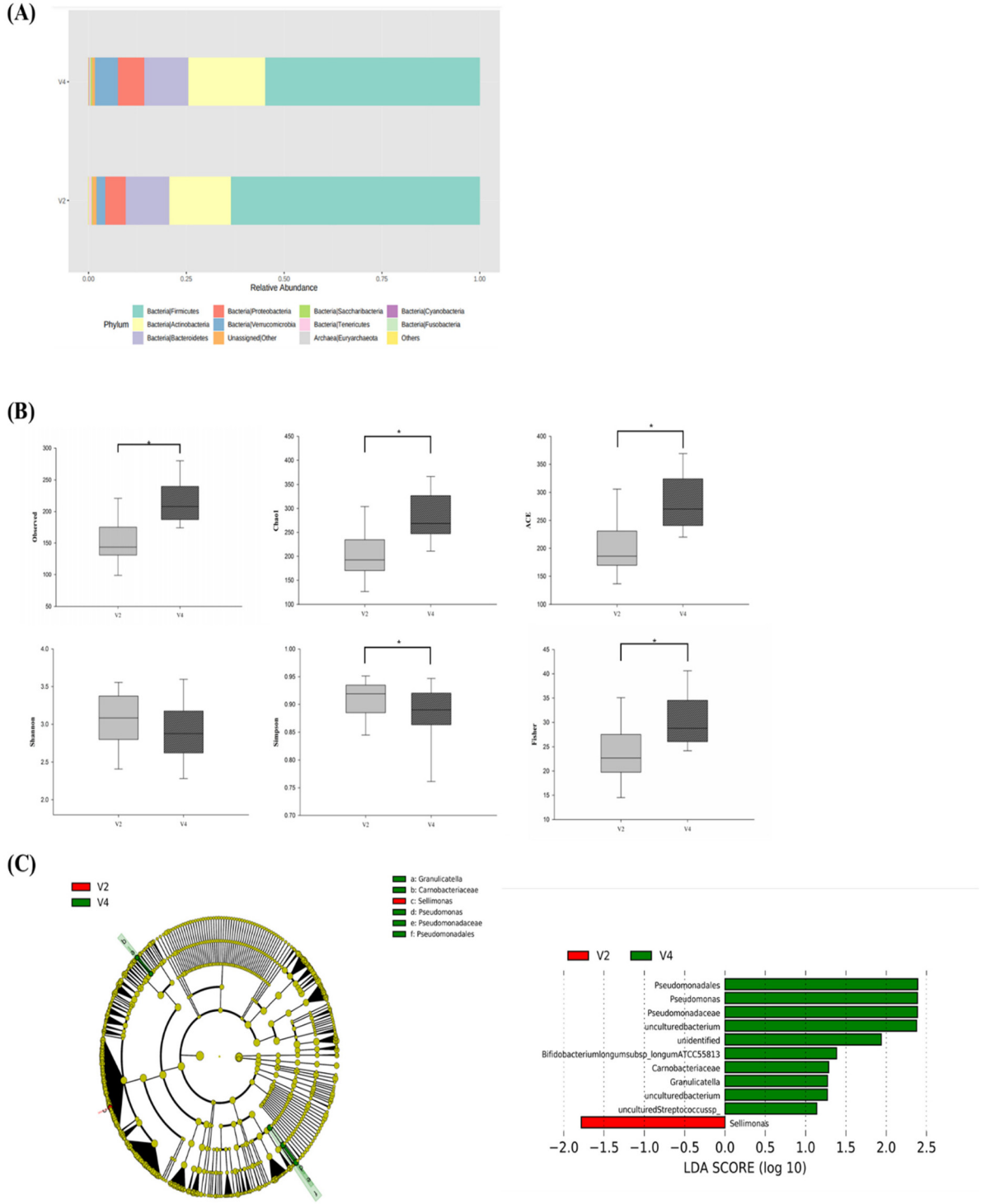

Fig. 2 (A) Altered microbiota composition after the ingestion of CWE, analyzed at the phylum level. (B) Alpha-diversity analysis was performed at the species level based on paired-sample *t* tests and the Wilcoxon rank sum test. **p* < 0.05. (C) LEfSe cladogram shows differences in the compositions of the microbiota from the phylum to the genus level before and after CWE ingestion. The *Y* axis represents the taxa with significant differences, while the *X* axis visually depicts the LDA score of the corresponding taxa by a bar chart (LDA score ≥1).

Pseudomonas, *unidentified Bifidobacterium*, *Bifidobacterium longum ATCC 55813*, *uncultured Granulicatella*, and *uncultured Streptococcus* were significantly increased (LDA = 2.38, 1.94, 1.38, 1.27, 1.14).

The correlation between biomarkers and changes in the gut microbiota was analyzed by Spearman's method. Fig. 3 shows the relationship between the two variables. Blue indicates a negative correlation, and red indicates a positive correlation.

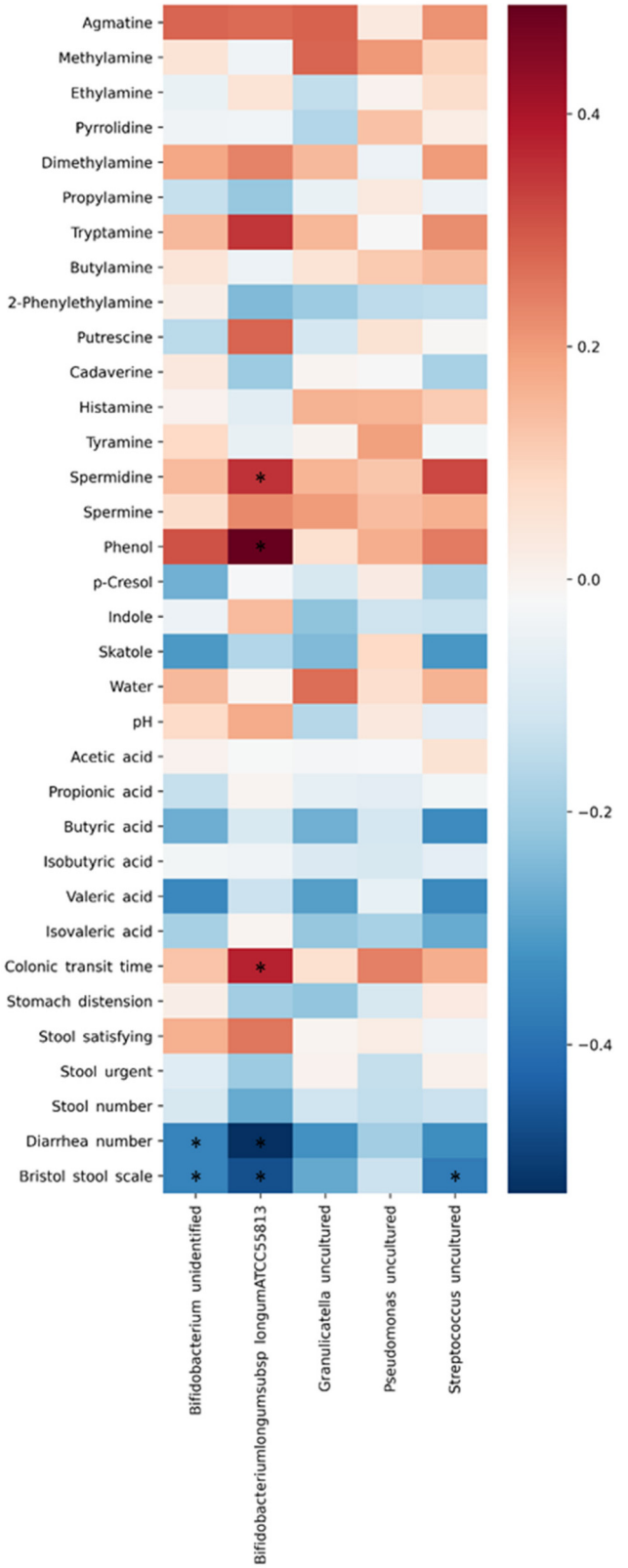

Fig. 3 Correlation analysis between the gut microbiota and biomarkers in feces based on Spearman's method. Blue indicates a negative correlation, and red shows a positive correlation. *$p < 0.05$.

The uncultured species of *Bifidobacterium*, which increased after the ingestion of CWE, showed a negative correlation with an improvement in the number of bowel movements with diarrhea and the Bristol stool scale score ($r = -0.359, -0.359$; $p < 0.05$). *Bifidobacterium longum ATCC 55813* showed a positive correlation with the amounts of phenol and spermidine and CTT ($r = 0.350, 0.494, 0.376$; $p < 0.05$). Conversely, *Bifidobacterium longum ATCC 55813* showed a negative correlation between the number of bowel movements with diarrhea and the Bristol stool scale score ($r = -0.528, -0.466$; $p < 0.05$). The uncultured species of *Streptococcus* showed a correlation with an improvement in the Bristol stool scale score ($r = -0.373$; $p < 0.05$).

Discussion

Cinnamon is a plant that has been used for a long time due to its unique fragrance. In a previous study, *C. cassia* was shown to alleviate inflammation and inhibit specific microbes.[15,23] *C. cassia* is spicy but bitter in taste and high in coumarin.[24] Moreover, coumarin and cinnamic acid, a component of cinnamon, can reduce the inflammatory response by inflammatory cytokines.[13,14] Hot water extraction is an efficient way to extract coumarin and cinnamic acid from *C. cassia*.[25] Hot water extraction also has the advantage of being less toxic than ethanol extraction.[26] Therefore, in this study, the effects of CWE prepared through the hot water extraction method on relieving symptoms of diarrhea were investigated through a clinical trial.

CTT is a representative indicator of diarrhea and plays an important role in the formation of metabolites and microbiota alterations.[27] Improved CTT in the transverse colon increases SCFAs.[28] SCFAs strengthen junctional complexes and upregulate tight junction assembly by engaging the 5′ adenosine monophosphate-activated protein kinase pathway.[29] CTT and isobutyric acid were significantly increased in the CWE group. Isobutyric acid is a branched-chain fatty acid produced in the process of fermenting branched-chain amino acids, such as valine, leucine, and isoleucine, by the intestinal flora.[30] This finding suggests the possibility that the increased isobutyric acid resulting from CWE ingestion might improve gut health and alleviate inflammation. CWE also decreased fecal indole concentrations. Indole is also known as an interspecies signaling molecule, and the composition of the gut microbiota can control the indole concentration.[31] The amount of agmatine formed by the intestinal microflora is an important physiological source of agmatine in human organisms. Various types of intestinal bacteria differ greatly in their ability to form agmatine. Therefore, the amount of absorbable agmatine should vary considerably depending on the composition of the bacterial flora.[32]

The microbial phylum ratio is associated with gut health indicators such as alpha diversity. CWE decreased the Firmicutes/Bacteroidetes ratio, which can be seen as a marker of improved alpha diversity in subjects.[33] Increased diversity of

the gut microbiota leads to resistance to perturbations, such as inflammatory responses, antibiotics, and external stimuli.[34] LEfSe analysis showed that CWE ingestion significantly increased *Bifidobacterium*, *Granulicatella*, *Streptococcus*, and *Pseudomonas* and decreased *Sellimonas* (LDA score $\geq$ 1.0). *Streptococcus* and *Pseudomonas* can biosynthesize spermidine by catabolizing agmatine during arginine and proline metabolism.[35,36] Increased spermidine production by the microbiota improves permeability and suppresses the inflammatory response.[37] Some *Pseudomonas* species, such as *P. aeruginosa*, have the ability to degrade indoles and enhance their biofilms in indole-rich environments.[38,39] This fact suggests that the decreased fecal indole concentration through the ingestion of cinnamon is due to an increase in the genus *Pseudomonas*. However, there was no significant difference observed in the correlation analysis, so verification through future studies will be needed.

Correlation analyses between specific microbes and stool contents and functional biomarkers, such as an increase in CTT and a decrease in the number of bowel movements with diarrhea and the Bristol stool scale score, showed a positive correlation with uncultured species of *Bifidobacterium* and *Bifidobacterium longum ATCC 55813*. Additionally, *Bifidobacterium longum ATCC 55813* showed a positive correlation with spermidine and phenol in feces. *Bifidobacterium longum* has the ability to produce spermidine *via* an unknown pathway.[40] Studies have reported that *Bifidobacterium longum* can significantly reduce diarrhea in children.[41] Uncultured species of *Streptococcus* showed a negative correlation with the Bristol stool scale score, and no significant correlation was found in the uncultured species of *Pseudomonas* and *Granulicatella*. These findings demonstrate that the intestinal microbiota, especially *Bifidobacterium*, was altered through the ingestion of CWE, which can improve gut health and alleviate diarrhea symptoms.

In conclusion, the ingestion of CWE improved CTT shortened by diarrhea. Moreover, CWE altered the biogenic amines, SCFAs, and indole in the stools of subjects with diarrhea by altering microorganism composition and metabolism in the human body. Coumarin and cinnamic acid were likely partially responsible for the effects of CWE, suggesting additive effects with other active compounds of CWE. In a previous study, polyphenol-rich cinnamon bark extract possessed antimicrobial activity against the tested Gram-positive bacteria, producing an increase in cinnamic acid *in vitro* digestion. In addition, cinnamon bark extract was found to exhibit antitumor effects on colon cell lines implemented in the presence of probiotic fermentation.[42] In this study, CWE, characterized by the presence of coumarin and cinnamic acid, showed the possibility of relieving diarrhea symptoms by reorganizing the intestinal environment. However, this study has certain limitations. The present study could not confirm the complex effects that may arise from the interaction of the intestinal environment and changes in the body state according to the metabolism of each microorganism. Further studies should investigate the potential effects of metabolic changes in each microbiota on colonic disease and evaluate the efficacy of CWE for diarrhea improvement with other drugs or alternatives, such as oligosaccharides.

Author contributions

Soo-yeon Park analyzed and interpreted the results and edited the draft. Yong Dae Kim performed the formal analysis and wrote the first draft of the manuscript. Min Seo Kim conceptualized the experiments and methodology. Ki-Tae Kim provided resources and performed investigations. Ji Yeon Kim reviewed and edited the draft, supervised the study, and provided project administration. All authors meet the CRediT criteria for authorship and have approved the final version of the manuscript for publication.

Conflicts of interest

There are no conflicts of interest to declare.

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