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Seaberry extract with ursolic acid improves anxiety about urinary dysfunction in Japanese adults

Tsuyoshi Takara[1], Kazuo Yamamoto[2], Naoko Suzuki[2], Hiroshi Shimoda[3] *

[1]Takara Clinic, Medical Corporation Seishinkai, 9F Taisei Building, 2-3-2, Higashi-Gotanda, Shinagawa-ku, Tokyo 141-0022, Japan; [2]ORTHOMEDICO Inc., 2F Sumitomo Fudosan Korakuen Bldg., 1-4-1 Koishikawa Bunkyo-ku, Tokyo, 112-0002 Japan; [3]Research and Development Division, Oryza Oil and Fat Chemical Co., Ltd., 1 Numata, Kitagata-cho, Ichinomiya, Aichi 493-8001, Japan

Corresponding author: Hiroshi Shimoda, PhD, Research and Development Division, Oryza Oil and Fat Chemical Co., Ltd., 1 Numata, Kitagata-cho, Ichinomiya, Aichi 493-8001, Japan

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ABSTRACT

Background: The seaberry is a yellow berry cultivated in China, Northern Europe, and West Asian countries. Numerous biological activities of seaberries have been reported, and we recently found that ursolic acid and a flavonoid in seaberry extract (SBE) suppressed the contraction of bladder muscle specimens and collagen gel containing bladder smooth muscle cells. However, the influence of SBE on urinary problems of Japanese adults has not been investigated. Therefore, we conducted a clinical trial of SBE supplementation in Japanese subjects with mild urinary dysfunction.

Methods: We examined the effect of SBE (200 or 400 mg daily) containing ursolic acid (0.18 or 0.36 mg) in a randomized double-blind placebo-controlled study. Capsules containing SBE or placebo were administered for 8 weeks to Japanese men and women with mild urinary dysfunction. After 4 and 8 weeks of treatment, urinary symptoms were evaluated by using the King's Health Questionnaire (KHQ) and the overactive bladder syndrome score (OABSS).

Results: There was significant improvement of the KHQ emotion domain score after intake of 400 mg/day of SBE for 8 weeks compared with placebo. The answers to "Does your bladder problem make you depressed?" and "Does your bladder problem make you feel bad about

yourself?" were significantly improved by SBE (400 mg/day) at 8 weeks. In the OABSS, the item "How often do you have a sudden desire to urinate, which is difficult to defer?" was significantly improved by SBE compared with placebo. Laboratory tests did not reveal any abnormalities suggesting adverse effects SBE.

Conclusion: Intake of SBE (400 mg/day for 8 weeks) improved several emotional parameters related to urinary dysfunction. SBE may be useful for reducing moderate urinary symptoms.

Keywords: King's health questionnaire, overactive bladder syndrome score, seaberry, ursolic acid, overactive bladder, urination, micturition

BACKGROUND

Overactive bladder (OAB) is a type of urinary dysfunction in which pain on micturition and frequent urination are typical symptoms [1]. For treatment of OAB, medications such as anti-chorinergics [2, 3], β_3-adnergic agonists [4], flavoxate [5], and trospium [6] are prescribed, while dietary supplements for urinary dysfunction include pumpkin seed oil [7], saw palmetto oil [8], and isosamidin from *Peucedanum japonicum* [9]. There have been few clinical studies of dietary supplements for OAB, even though more than 8 million Japanese people have this condition [10].

Seaberry (*Hippophae rhamnoides*) is a fruit cultivated in China, Mongolia, Canada, and various northern and eastern European countries. Clinical trials have demonstrated the hepatoprotective [11, 12] and anti-inflammatory [13, 14] effects of seaberry, in addition to its usefulness for eye care [15, 16] and weight loss [17, 18]. We recently found that seaberry extract (SBE) and its constituents (ursolic acid and flavonol glucoside) suppressed cholinergic bladder smooth muscle contraction *in vitro* [19]. Accordingly, we conducted a clinical trial to assess the effect of SBE on anxiety in Japanese subjects with moderate OAB.

METHODS

Participants

To recruit subjects, 60 participants registered with the monitor bank of ORTHOMEDICO Inc. by answering several questionnaires. The registration criteria were an age of 40 years or older, either sex, and frequent urination (usually at least 8 times a day). Exclusion criteria are listed below:

1) Previous malignancy, heart failure, and myocardial infarction.
2) Current treatment for arrhythmia, hepatitis, nephritis, rheumatoid arthritis, cerebrovascular accident, diabetes, hyperlipidemia, hypertension, or other chronic diseases.
3) Current use of medications or dietary supplements.
4) History of severe allergic reactions to foods or medicines.
5) Pregnant or breastfeeding women and women wanting to become pregnant.
6) Persons currently participating in another clinical trial or who have participated within the previous 3 months.

7) Persons determined to be inappropriate for the study by the attending physician.

Forty-eight persons with relatively high King's Health Questionnaire (KHQ) scores [20] were selected after being confirmed to be suitable for the study by a physician (**Fig. 1**). Then 16 subjects were allocated to each of 3 groups by using software for Microsoft Excel (Statlight #11, Yukms Co. Ltd., Kawasaki, Japan). Grouping was based on the mean and standard deviation of the KHQ score, the male/female ratio, and the age. The subjects were asked to avoid overeating and overdrinking and to maintain a regular lifestyle during the study period. Before the day of testing, they were asked to avoid excessive intake of alcohol and excessive exercise, and they fasted from 6 hr prior to blood collection except for drinking water.

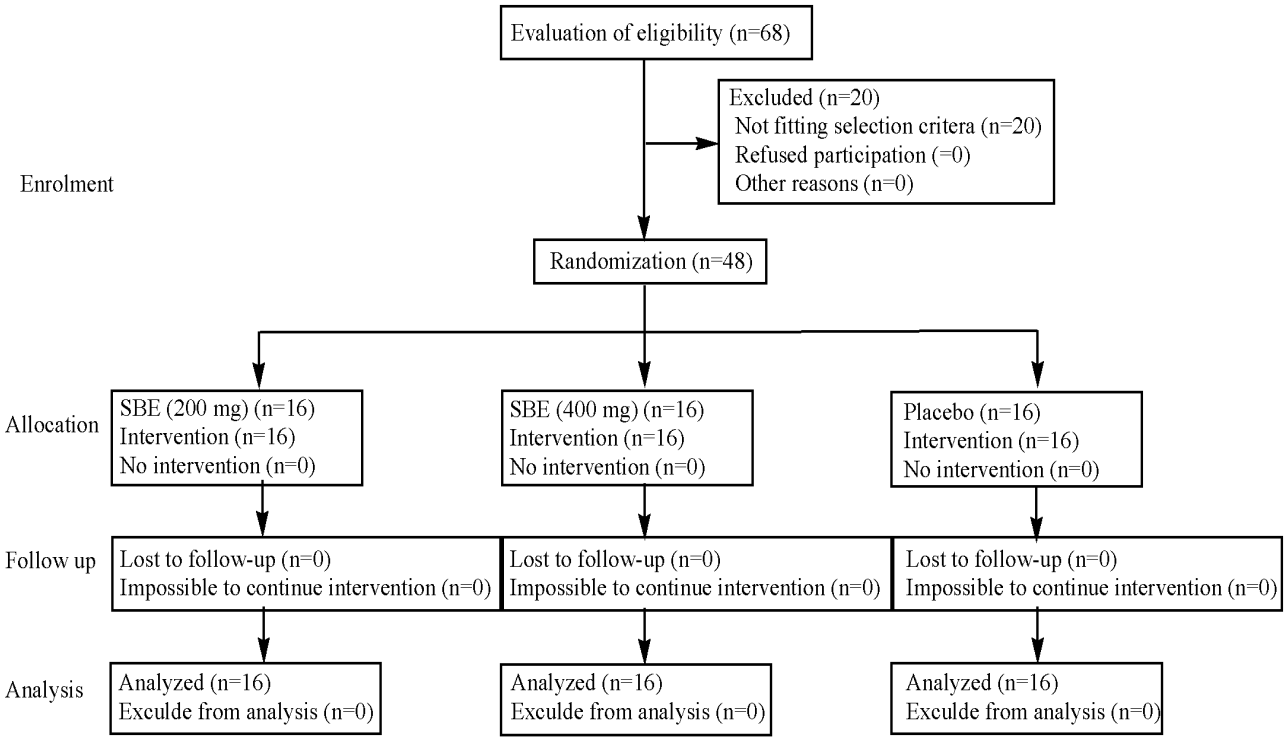

Figure 1. Flowchart showing the disposition of the subjects

Preparation and allocation of test supplements

The test supplements (indistinguishable brown capsules containing SBE or placebo) were provided by Oryza Oil & Fat Chemical Co., Ltd. The active capsules contained 200 mg of Seaberry Extract-P (seaberry extract powder with 0.09% ursolic acid, Oryza Oil & Fat Chemical Co. Ltd.), while the placebo capsules contained 200 mg of dextrin. Information about the allocation of the test medications was strictly protected by a third-party study treatment/allocation controller who was not directly involved in the study. The information was not disclosed to anybody until the subjects for analysis were determined at a clinical conference after completion of the study.

Study protocol

This randomized placebo-controlled double-blind study was carried out at Takara Clinic (Seishinkai Group, Tokyo), and statistical analysis was done by ORTHOMEDICO Inc. The

study was registered with the University Hospital Medical Information Network (UMIN000023486). It involved comparison among three groups receiving concurrent treatment. Subjects in the placebo group and the SBE (400 mg) group took one capsule (placebo or SBE respectively) after breakfast and after dinner. Subjects in the SBE (200 mg) group took one SBE capsule after breakfast and one placebo capsule after dinner. All subjects took the study capsules for 8 weeks and wrote a daily report about capsule ingestion, lifestyle, urination, and menstruation (only women).

Table 1. The King's Health Questionnaire (KHQ)

Question	Score	1	2	3	4	5
1. How would you describe your health at the present?		Very good	Good	Fair	Poor	Very poor
2. How much do you think your bladder problem affects your life?		Not at all	A little	Moderately	A lot	-
3. Role limitations		Not at all	Slightly	Moderately	A lot	-
a. Does your bladder problem affect your household tasks?		○	○	○	○	-
b. Does your bladder problem affect your job, or your normal daily activities outside the home?		○	○	○	○	-
4. Physical / Social limitations		Not at all	Slightly	Moderately	A lot	-
a. Does your bladder problem affect your physical activities?		○	○	○	○	-
b. Does your bladder problem affect your ability to travel?		○	○	○	○	-
c. Does your bladder problem limit your social life?		○	○	○	○	-
d. Does your bladder problem limit your ability to see and visit friends?		○	○	○	○	-
	Score	0	1	2	3	4
5. Personal relationships		Not applicable	Not at all	Slightly	Moderately	A lot
a. Does your bladder problem affect your relationship with your partner?		○	○	○	○	○
b. Does your bladder problem affect your sex life?		○	○	○	○	○
c. Does your bladder problem affect your family life?		○	○	○	○	○
	Score	1	2	3	4	-
6. Emotions		Not at all	Slightly	Moderately	Very much	-
a. Does your bladder problem make you feel depressed?		○	○	○	○	-
b. Does your bladder problem make you feel anxious or nervous?		○	○	○	○	-
c. Does your bladder problem make you feel bad about yourself?		○	○	○	○	-
	Score	1	2	3	4	-
7. Sleep / Energy		Never	Sometimes	Often	All the time	-
a. Does your bladder problem affect your sleep?		○	○	○	○	-
b. Does your bladder problem make you feel worn out and tired?		○	○	○	○	-
8. Do you do any of the following? If so, how much?		Never	Sometimes	Often	All the time	-
a. Wear pads to keep dry?		○	○	○	○	-
b. Be careful how much fluid you drink?		○	○	○	○	-
c. Change your underclothes because they get wet?		○	○	○	○	-
d. Worry in case you smell?		○	○	○	○	-

Table 2. Nine domains calculated from the KHQ

Question	Calculation
General health perception	(Score for Q1-1)/4×100
Incontinence impact	(Score for Q2-1)/3 ×100
Role limitations	[{(Sum of scores for Q3a and Q3b)-2}/6]×100
Physical limitations	[{(Sum of scores for Q4a and Q4b)-2}/6]×100
Social limitations	If score for Q5c > 1: [{(Sum of scores for Q4c, Q4d and Q5c)-3}/9] ×100 If score for Q5c is 0: [{(Sum of scores for Q4c and Q4d)-2}/6]×100
Personal relationships	If sum of scores for Q5a and 5b≥2: [{(Sum of scores for Q5a and Q5b)-2}/6]×100 If sum of scores for Q5a and Q5b=1: [{(Sum of scores for Q5a and Q5b)-1}/3]×100 If sum of scores for Q5a and Q5b=0: treat as a missing value
Emotions	[{(Sum of scores for Q6a, Q6b and Q6c)-3}/9]×100
Sleep/Energy	[{(Sums of scores for Q7a and Q7b)-2}/6]×100
Severity/coping	[{(Sum of scores for Q8a, Q8b, Q8c and Q8d)-4}/12]×100

Q: Questions in Table 1.

The primary outcome was the KHQ score, which was determined before treatment and after 4 or 8 weeks of treatment (Table 1). Nine QOL domains of the KHQ were calculated by the formulae shown in Table 2. The secondary outcome was the OABSS [21] (Table 3). Subjects completed the questionnaires and performed blood tests and urine collection before the start of treatment in addition to after 4 and 8 weeks of treatment. Measurement of physical parameters (height, body weight, body mass index (BMI), body fat percentage, blood pressure, and pulse rate) was performed on the same days, and the daily reports of the subjects were collected. Efficacy and safety of SBE were evaluated from the data obtained as described above.

Table 3. Overactive bladder syndrome score (OABSS)

Question	Frequency	Score
1. How many times do you typically urinate from waking in the morning until sleeping at night?	≤7	0
	8-14	1
	≥15	2
2. How many times do you typically wake up to urinate from sleeping at night until waking in the morning?	0	0
	1	1
	2	2
	≥3	3
3. How often do you have a sudden desire to urinate, which is difficult to defer?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5
4. How often do you leak urine because you cannot defer the sudden desire to urinate?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5

Laboratory tests

Analysis of blood and urine was performed by LSI Medience Corporation (Tokyo). A venous blood sample was collected from an arm vein and the following parameters were determined for assessment of safety.

Hematology parameters: Red blood cell count, leukocyte count, hemoglobin, hematocrit, platelet count, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).

Biochemical parameters: total protein, total bilirubin, urea N, creatinine, uric acid, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride, free fatty acids, blood glucose, alkaline phosphatase (ALP), creatinine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GTP), amylase, lactate dehydrogenase (LDH), Na, K, Cl, Ca, Fe, and inorganic phosphorus (IP).

Additionally, urine samples were collected for the qualitative evaluation of protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood.

Ethics, adherence, and compliance

This study was performed according to the Declaration of Helsinki (2013 revision) and was carried out in conformity with ethical considerations. The Ethics Committee of ORTHOMEDICO Inc. (an insurance company manager, 2 physicians, 3 nurses, and a lawyer) was convened to deliberate on the ethicality and appropriateness of the study protocol. This study was implemented according to the protocol approved by the Ethics Committee, and any substantial deviations from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before giving consent to participation.

Investigation of adverse events

Each adverse event was evaluated to determine the causal relationship with the test substance. Adverse events were defined as clinically significant new physical abnormalities or symptoms that occurred during the study period. If an adverse event occurred, the physician provided appropriate treatment and decided whether the study should be continued. The causal relationship with study treatment was judged according to the following criteria.

1. Unrelated. The cause of the event is not considered to be related to the test substance.
2. Probably unrelated. There is no chronological relationship between the event and use of the test substance.
3. Probably related. There is a possible chronological relationship between the event and use of the test substance.
4. Related. There is an obvious chronological relationship between the event and use of the test substance.
5. Unclear. It cannot be determined whether or not the event was caused by the test substance.

Statistical analysis

Background factors and blood parameters are reported as the mean and SD, while KHQ and OABSS scores are given as the median and interquartile range. Urine parameters are reported

as integers. For background factors and blood parameters, Dunnett's test was performed to compare values before treatment with those after 4 or 8 weeks of treatment. KHQ and OABSS scores were analyzed by the Wilcoxon signed-rank test for comparison between before and after treatment or Steel's test for comparisons among groups. SPSS ver. 23.0 software (Japan IBM) and R ver. 3.3.1 software (The R foundation) were used for these analyses.

RESULTS

Study performance

Table 4 shows the profile of each group. None of the subjects took less than 90% of the prescribed capsules during the study period, so the data on all subjects were used for analysis.

Table 4. Profile of the participants.

	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)
Sex (male/female)	7/9	7/9	5/11
Age (years)	51.1±8.2	51.4±9.6	51.0±6.8
Height (cm)	161.8±8.1	163.7±5.2	163.0±8.8
Body weight (kg)	55.8±10.6	65.3±16.2	56.6±12.0
BMI (kg/m^2)	21.2±2.7	24.3±5.7	21.1±2.7
Body fat ratio (%)	20.9±4.7	24.6±8.1	21.3±4.3
Systolic pressure (mmHg)	128.1±19.9	124.1±19.4	127.0±20.2
Diastolic pressure (mmHg)	80.8±12.9	78.3±13.7	81.7±12.9
Pulse rate (bpm)	72.4±8.7	72.0±11.2	74.8±8.3

Data are represented as the mean ±SD. There were no significant differences between the placebo group and the SBE groups.

KHQ score and OABSS

(1) KHQ score

Among the nine KHQ domains, the placebo group revealed significant decreases of the scores for "incontinence impact," "role limitations," and "physical limitations" after 8 weeks (Table 5). After intake of SBE (200 mg) for 4 weeks, scores for "incontinence impact," "sleep/energy," and "severity/coping" were significantly improved compared to before treatment. Additionally, there was a significant reduction of the "sleep/energy" score after 8 weeks of treatment with SBE (200 mg). After intake of SBE (400 mg) for 8 weeks, the scores for "incontinence impact,"

"role limitations," "physical limitations," and "severity/coping" were significantly improved compared to before treatment, and the "emotions" score also demonstrated significant improvement compared to the placebo group.

Table 5. Changes of the nine domains in the KHQ

Domain	Week	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)
General health perception	0	25.0 (25.0-25.0)	25.0 (25.0-31.3)	25.0 (25.0-50.0)
	4	25.0 (25.0-50.0)	25.0 (25.0-50.0)	25.0 (25.0-50.0)
	8	25.0 (25.0-25.0)	25.0 (18.8-50.0)	25.0 (25.0-31.3)
Incontinence impact	0	33.3 (33.3-66.7)	33.3 (33.3-66.7)	33.3 (33.3-33.3)
	4	33.3 (33.3-33.3)	33.3 (25.0-33.3)†	33.3 (33.3-33.3)
	8	33.3 (33.3-33.3)†	33.3 (33.3-33.3)	33.3 (25.0-33.3)†
Role limitations	0	33.3 (16.7-54.2)	25.0 (16.7-37.5)	33.3 (26.7-33.3)
	4	16.7 (16.7-33.3)†	16.7 (0.0-20.8)	16.7 (0.0-20.8)†
	8	16.7 (0.0-33.3)†	16.7 (12.5-33.3)	16.7 (0.0-16.7)††
Physical limitations	0	33.3 (29.2-50.0)	33.3 (16.7-50.0)	16.7 (16.7-50.0)
	4	25.0 (16.7-54.2)	16.7 (0.0-33.3)	16.7 (12.5-33.3)
	8	16.7 (12.5-33.3)†	16.7 (0.0-33.3)	16.7 (0.0-33.3)†
Social limitations	0	11.1 (0.0-36.1)	0.0 (0.0-22.2)	0.0 (0.0-16.7)
	4	5.6 (0.0-25.0)	0.0 (0.0-0.0)	0.0 (0.0-11.1)
	8	5.6 (0.0-25.0)	0.0 (0.0-11.1)	0.0 (0.0-2.8)
Personal relationships	0	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-16.7)
	4	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-16.7)
	8	0.0 (0.0-8.3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Emotions	0	33.3 (11.1-44.4)	33.3 (11.1-33.3)	11.1 (11.1-33.3)
	4	22.2 (11.1-33.3)	11.1 (0.0-33.3)	11.1 (8.3-25.0)
	8	33.3 (1.1.-44.4)	11.1 (0.0-33.3)	11.1 (0.0-11.1)††, *
Sleep/energy	0	16.7 (16.7-33.3)	16.7 (16.7-33.3)	16.7 (12.5-33.3)
	4	16.7 (0.0-37.5)	16.7 (0.0-16.7)†	25.0 (12.5-33.3)
	8	16.7 (16.7-20.8)	16.7 (0.0-33.3)	16.7 (0.0-33.3)
Severity/coping	0	26.7 (6.7-33.3)	13.3 (13.3-35.0)	16.7 (6.7-28.3)
	4	13.3 (6.7-33.3)	6.7 (6.7-21.7)†	13.3 (6.7-21.7)
	8	13.3 (11.7-28.3)	10.0 (0.0-21.7)††	10.0 (6.7-20.0)†

Data are represented as the median and interquartile range. Daggers denote significant differences vs. before ingestion at †: $p<0.05$, ††: $p<0.01$. Asterisk indicates a significant difference from placebo at *: $p<0.05$.

Table 6a. Changes of individual items in the KHQ

Item	Week	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)
Q1. How would you describe your health at the present?	0	2.0 (2.0-2.0)	2.0 (2.0-2.3)	2.0 (2.0-3.0)
	4	2.0 (2.0-3.0)†	2.0 (2.0-3.0)	2.0 (2.0-3.0)
	8	2.0 (2.0-2.0)	2.0 (1.8-3.0)	2.0 (2.0-2.3)
Q2. How much do you think your bladder problem affects your life?	0	2.0 (2.0-3.0)	2.0 (2.0-3.0)	2.0 (2.0-2.0)
	4	2.0 (2.0-2.0)	2.0 (1.8-2.0)†	2.0 (2.0-2.0)
	8	2.0 (2.0-2.0)†	2.0 (2.0-2.0)	2.0 (1.0-2.0)†
Q3a. Does your bladder problem affect your household tasks?	0	2.0 (1.0-2.0)	1.5 (1.0-2.0)	2.0 (1.0-2.0)
	4	1.0 (1.0-2.0)†	1.0 (1.0-2.0)	1.0 (1.0-1.3)
	8	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)
Q3b. Does your bladder problem affect your job, or your normal daily activities outside the home?	0	2.0 (2.0-3.0)	2.0 (2.0-3.0)	2.0 (2.0-2.0)
	4	2.0 (2.0-2.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)
	8	2.0 (1.0-2.0)†	2.0 (1.8-2.0)	1.5 (1.0-2.0)†
Q4a. Does your bladder problem affect your physical activities?	0	1.5 (1.0-2.3)	1.5 (1.0-2.0)	1.0 (1.0-2.0)
	4	1.5 (1.0-2.0)	1.5 (1.0-2.0)	1.0 (1.0-2.0)
	8	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)
Q4b. Does your bladder problem affect your ability to travel?	0	2.5 (2.0-3.0)	2.0 (2.0-3.0)	2.0 (2.0-2.3)
	4	2.0 (2.0-3.0)	2.0 (1.0-2.0)†	2.0 (1.0-2.0)
	8	2.0 (1.8-3.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)
Q4c. Does your bladder problem limit your social life?	0	2.0 (1.0-2.3)	1.0 (1.0-2.0)	1.0 (1.0-1.3)
	4	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
	8	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.3)
Q4d. Does your bladder problem limit your ability to see and visit friends?	0	1.0 (1.0-2.3)	1.0 (1.0-2.0)	1.0 (1.0-2.0)
	4	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
	8	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Q5a. Does your bladder problem affect your relationship with your partner?	0	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.3)
	4	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.3)
	8	1.0 (1.0-1.3)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Q5b. Does your bladder problem affect your sex life?	0	1.0 (0.8-1.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)
	4	1.0 (0.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.3)
	8	1.0 (0.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Q5c. Does your bladder problem affect your family life?	0	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)
	4	1.0 (1.0-1.3)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
	8	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Q6a. Does your bladder problem make you depressed?	0	2.0 (1.0-2.0)	2.0 (1.8-2.0)	1.0 (1.0-2.0)
	4	2.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)
	8	2.0 (2.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)*
Q6b. Does your bladder problem make you feel anxious or nervous?	0	2.0 (2.0-2.3)	2.0 (1.8-2.0)	2.0 (2.0-2.0)
	4	2.0 (1.8-2.0)	1.0 (1.0-2.0) †	2.0 (1.8-2.0)
	8	2.0 (1.8-3.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)†
Q6c. Does your bladder problem make you feel bad about yourself?	0	2.0 (1.0-3.0)	2.0 (1.0-2.0)	1.0 (1.0-2.0)
	4	1.5 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.3)
	8	2.0 (1.0-2.0)	2.0 (1.0-2.0)	1.0 (1.0-1.0)**

Data are represented as the median and interquartile range. Daggers denote significant differences vs. before ingestion at †: $p<0.05$. Asterisks indicate significant differences from placebo at *: $p<0.05$, **: $p<0.01$.

Table 6b. Changes of individual items in the KHQ

Question	Week	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)	Question	Week	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)
Q7a. Does your bladder problem affect your sleep?	0	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (1.8-2.0)	Q8c. Change your underclothes because they get wet?	0	1.5 (1.0-2.0)	1.0 (1.0-1.3)	1.5 (1.0-2.0)
	4	2.0 (1.0-2.3)	2.0 (1.0-2.0)†	2.0 (1.8-2.3)		4	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (10-1.3)
	8	2.0 (1.8-2.0)	2.0 (1.0-2.0)	2.0 (1.0-2.3)		8	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.3)
Q7b. Does your bladder problem make you feel worn out and tired?	0	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	Q8d. Worry in case you smell?	0	1.0 (1.0-2.0)	2.0 (1.0-2.0)	1.0 (1.0-2.0)
	4	1.0 (1.0-2.0)	1.0 (1.0-1.3)	1.0 (1.0-2.0)		4	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)
	8	1.0 (1.0-2.0)	1.0 (1.0-1.3)	1.0 (1.0-1.0)		8	1.0 (1.0-2.0)	1.0 (1.0-1.3)†	1.0 (1.0-1.0)
Q8a. Wear pads to keep dry?	0	1.0 (1.0-1.3)	1.0 (1.0-1.5)	1.0 (1.0-1.3)	Q8e. Get embarrassed because of your bladder problem?	0	2.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)
	4	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)		4	1.5 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.3)
	8	1.0 (1.0-1.0)	1.0 (1.0-1.3)	1.0 (1.0-1.0)		8	1.0 (1.0-2.0)	1.0 (1.0-1.3)	1.0 (1.0-1.0)
Q8b. Be careful how much fluid you drink?	0	2.5 (2.0-3.0)	2.5 (2.0-3.0)	2.0 (2.0-3.0)					
	4	2.0 (2.0-2.3)	2.0 (1.0-2.0)	2.0 (2.0-3.0)					
	8	2.0 (2.0-3.0)	2.0 (1.0-2.3)†	2.0 (1.8-2.3)					

Data are represented as the median and interquartile range. Daggers denote significant differences vs. before ingestion at †: $p<0.05$.

When the individual items of the KHQ were assessed (Table 6), there was no improvement of the median values (interquartile range) for any of the questions after 4 or 8 weeks of placebo treatment. In the SBE (200 mg) group, the scores for Q2, Q4b, Q6b, and Q7a showed improvement after 4 weeks compared to before treatment, while the scores for Q8b and Q8d were significantly improved after 8 weeks. In the SBE (400 mg) group, there was significant improvement of the scores for Q2, Q3b, and Q6b after 8 weeks. The scores for Q6a ("Does your bladder problem make you depressed?") and Q6c ("Does your bladder problem make you feel bad about yourself?") showed significant improvement in the SBE (400 mg) group compared to the placebo group.

(2) OABSS

As shown in Table 7, the total OABSS score and the score for Q2 improved at 4 and 8 weeks in the SBE (200 mg) group. In the SBE (400 mg) group, there was significant reduction of the score for Q3 ("How often do you have a sudden desire to urinate, which is difficult to defer?") after 8 weeks compared to the placebo group.

Safety parameters

(1) Blood pressure and pulse rate

Assessment of the blood pressure and pulse rate revealed a significant decrease of systolic pressure after 4 weeks in the placebo group. In the SBE groups, there were no significant changes of the blood pressure and pulse rate after 4 and 8 weeks of treatment (Table 8).

(2) Laboratory parameters

Hematology and biochemical parameters are displayed in **Tables 8 and 9** respectively. In the placebo group, MCV and total protein were slightly decreased after 4 and 8 weeks. In the SBE (200 mg/day) group, the red blood cell count was significantly increased after 8 weeks of treatment and a similar change was observed in the SBE (400 mg/day) group at 8 weeks. Amylase and potassium were increased in the SBE (200 mg/day) group at 8 weeks, while IP was decreased. There were also significant increases of hemoglobin, LDL-cholesterol, γ-GTP, amylase, and potassium in the SBE (400 mg/day) group at 8 weeks, while triglyceride and IP were decreased. However, all of these changes were small and within the normal range.

(3) Urinalysis

Occult blood was detected in the urine of several subjects in each group (**Table 10**). However, investigation by the physician in charge demonstrated that these events were not induced by study treatment.

Table 7. Changes of the OABSS

Question	Week	Placebo (n = 16)	SBE (200 mg) (n = 16)	SBE (400 mg) (n = 16)
Total OABSS	0	4.0 (3.0-5.0)	3.5 (3.0-6.0)	3.0 (2.8-4.0)
	4	3.0 (2.0-4.3)	3.0 (2.0-5.0)††	2.0 (2.0-3.3)
	8	3.0 (2.8-4.3)	2.5 (2.0-4.0)††	2.0 (1.8-3.0)
1. How many times do you typically urinate from waking in the morning until sleeping at night?	0	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
	4	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
	8	1.0 (1.0-1.0)	1.0 (0.0-1.0)	1.0 (1.0-1.0)
2. How many times do you typically wake up to urinate from sleeping at night until waking in the morning?	0	1.0 (0.0-1.0)	1.0 (1.0-1.0)	1.0(0.8-1.0)
	4	1.0 (0.0-1.0)	1.0 (0.0-1.0)†	1.0 (0.8-1.0)
	8	1.0 (0.0-1.0)	1.0 (0.8-1.0)†	1.0 (0.8-1.0)
3. How often do you have a sudden desire to urinate, which is difficult to defer?	0	1.5 (1.0-2.0)	1.5 (1.0-2.0)	1.0 (0.8-1.3)
	4	1.0 (0.0-2.0)	1.0 (1.0-2.0)	0.0 (0.0-2.0)
	8	1.0 (1.0-2.0)	1.0 (0.0-1.3)	0.0 (0.0-1.0)*
4. How often do you leak urine because you cannot defer the sudden desire to urinate?	0	0.0 (0.0-0.3)	0.0 (1.0-1.0)	0.0 (0.0-1.0)
	4	0.0 (0.0-0.0)	0.0 (0.0-1.0)	0.0 (0.0-0.0)
	8	0.0 (0.0-0.0)	0.5 (0.0-1.0)	0.0 (0.0-0.0)

Data are represented as the median and interquartile range. Daggers denote significant differences vs. before ingestion at †: $p<0.05$, ††: $p<0.01$. Asterisk indicates a significant difference from placebo at *: $p<0.05$

Table 8. Changes of the blood pressure, pulse rate, and hematology parameters

	Before ingestion	After 4 weeks of ingestion	After 8 weeks of ingestion	Standard value
Systolic pressure (mmHg)				
Placebo	128.1±19.9	121.9±15.2†	125.1±18.1	<125
SBE (200 mg/day)	124.1±19.4	122.8±20.4	124.1±18.7	
SBE (400 mg/day)	127.0±20.2	123.0±20.1	123.8±21.9	
Diastolic pressure (mmHg)				
Placebo	80.8±12.9	79.4±11.3	78.9±11.9	<85
SBE (200 mg/day)	78.3±13.7	76.8±11.8	77.6±11.5	
SBE (400 mg/day)	81.7±12.9	78.9±15.7	77.9±14.8	
Pulse rate (beats/min)				
Placebo	72.4±8.7	79.0±12.5	73.6±9.0	
SBE (200 mg/day)	72.0±11.2	74.5±16.1	72.3±11.8	
SBE (400 mg/day)	74.8±8.3	72.9±10.2	74.0±12.6	
Red blood cells ($\times 10^4$cells/μL)				
Placebo	458±38	462±37	462±43	Male 427-570
SBE (200 mg/day)	460±45	466±49	469±50†	Female 327-500
SBE (400 mg/day)	438±36	446±33	447±39†	
Leukocytes (cells/μL)				
Placebo	5125±944	5056±1159	4956±1033	Male 3900-9800
SBE (200 mg/day)	5700±1548	6025±2566	5662±1409	Female 3500-9100
SBE (400 mg/day)	5138±1270	5081±1094	4981±1173	
Hemoglobin (g/dL)				
Placebo	14.0±1.1	14.0±1.3	14.0±1.4	Male 13.5-17.6
SBE (200 mg/day)	13.9±1.5	14.0±1.6	14.2±1.6	Female 11.3-15.2
SBE (400 mg/day)	13.2±1.3	13.3±1.3	13.5±1.3†	
Hematocrit (%)				
Placebo	44.4±3.1	44.0±3.5	43.8±3.8	Male 39.8-51.8
SBE (200 mg/day)	44.2±4.2	44.4±4.6	44.6±4.5	Female 33.4-44.9
SBE (400 mg/day)	41.8±3.5	42.4±3.5	42.8±3.8	
Platelets ($\times 10^4$cells/μL)				
Placebo	27.4±8.1	28.4±7.1	28.0±8.3	13.0-36.9
SBE (200 mg/day)	25.4±5.5	25.7±4.0	26.2±4.7	
SBE (400 mg/day)	26.2±4.8	27.1±5.1	28.0±6.0	
MCV (fL)				
Placebo	96.9±4.2	95.4±3.7†	94.9±3.4††	Male 83-102
SBE (200 mg/day)	96.3±4.6	95.2±3.1	95.2±3.9	Female 79-100
SBE (400 mg/day)	95.7±5.4	95.3±5.6	95.7±5.2	
MCH (pg)				
Placebo	30.7±1.4	30.3±1.5†	30.4±1.4	Male 28.0-34.6
SBE (200 mg/day)	30.3±1.7	30.1±1.5	30.3±1.8	Female 26.3-34.3
SBE (400 mg/day)	30.1±2.0	29.9±2.0	30.2±1.9	
MCHC (%)				
Placebo	31.6±0.8	31.7±0.8	32.1±0.9	Male 31.6-36.6
SBE (200 mg/day)	31.5±0.7	31.5±0.8	31.8±0.7	Female 30.7-36.6
SBE (400 mg/day)	31.5±0.8	31.4±0.9	31.5±0.7	

Data are represented as the mean ±SD (n=16). Daggers denote significant differences vs. before ingestion at †: $p<0.05$, ††: $p<0.01$.

Table 9a. Changes of biochemical parameters

	Before ingestion	After 4 weeks of ingestion	After 8 weeks of ingestion	Standard value
Total protein (g/dL)				
Placebo	7.4±0.5	7.3±0.4 †	7.3±0.5	6.7-8.3
SBE (200 mg/day)	7.2±0.4	7.1±0.4	7.2±0.4	
SBE (400 mg/day)	7.1±0.3	7.1±0.4	7.1±0.3	
Total bilirubin (mg/dL)				
Placebo	0.85±0.24	0.75±0.26	0.69±0.21	3.8-5.3
SBE (200 mg/day)	0.80±0.15	0.79±0.18	0.79±0.17	
SBE (400 mg/day)	0.91±0.34	0.83±0.23	0.86±0.24	
Urea N (mg/dL)				
Placebo	12.0±3.0	12.3±3.2	12.9±3.3	8-22
SBE (200 mg/day)	12.5±3.0	12.2±2.7	12.9±3.4	
SBE (400 mg/day)	13.2±4.0	13.5±4.5	14.1±4.6	
Creatinine (mg/dL)				
Placebo	0.72±0.14	0.68±0.13	0.69±0.13	Male 0.61-1.04
SBE (200 mg/day)	0.72±0.13	0.72±0.13	0.70±0.13	Female 0.47-0.79
SBE (400 mg/day)	0.70±0.15	0.69±0.14	0.69±0.16	
Uric acid (mg/dL)				
Placebo	5.0±1.4	5.0±1.4	5.2±1.4	Male 3.7-7.0
SBE (200 mg/day)	5.3±1.4	5.1±1.3	5.2±1.1	Female 2.5-7.0
SBE (400 mg/day)	4.2±1.3	4.2±1.3	4.3±1.4	
Total cholesterol (mg/dL)				
Placebo	218±22	213±21	220±29	130-219
SBE (200 mg/day)	205±28	205±33	209±34	
SBE (400 mg/day)	207±28	208±30	215±37	
LDL-cholesterol (mg/dL)				
Placebo	123±23	115±25	128±27	70-139
SBE (200 mg/day)	116±27	117±30	121±31	
SBE (400 mg/day)	116±28	119±26	128±33††	
HDL-cholesterol (mg/dL)				
Placebo	80±17	77±20	76±22	Male 40-86
SBE (200 mg/day)	70±14	69±13	69±16	Female 40-96
SBE (400 mg/day)	74±27	73±23	72±21	
Triglyceride (mg/dL)				
Placebo	73±24	139±28	86±34	35-149
SBE (200 mg/day)	93±62	99±56	98±53	
SBE (400 mg/day)	91±44	81±39	71±22††	
Free fatty acid (mEq/dL)				
Placebo	0.61±0.27	0.63±0.29	0.58±0.22	0.10-0.85
SBE (200 mg/day)	0.67±0.25	0.63±0.22	0.65±0.21	
SBE (400 mg/day)	0.60±0.20	0.57±0.20	0.59±0.18	
Blood glucose (mg/dL)				
Placebo	81±6	82±6	83±8	70-109
SBE (200 mg/day)	86±11	85±12	85±17	
SBE (400 mg/day)	79±8	79±9	82±8	
ALP (U/L)				
Placebo	184±42	195±45	198±46	110-360
SBE (200 mg/day)	185±44	180±43	185±43	
SBE (400 mg/day)	188±54	197±55	194±45	

Data are represented as the mean ±SD (n=16). Daggers denote significant differences vs. before ingestion at †: $p<0.05$ and ††: $p<0.01$.

Table 9b. Changes of biochemical parameters

CK (U/L)					Na (mEq/L)				
Placebo	104±45	101±57	89±33	Male 50-250	Placebo	141±2	142±2	141±1	137-147
SBE (200 mg/day)	101±46	107±58	96±40	Female 45-210	SBE (200 mg/day)	141±2	141±2	141±1	
SBE (400 mg/day)	102±39	100±29	114±64		SBE (400 mg/day)	142±2	142±2	141±2	
AST (U/L)					K (mEq/L)				
Placebo	23.4±6.8	24.2±7.9	22.7±4.4	10-40	Placebo	4.0±0.5	4.1±0.4	4.2±0.4	3.5-5.0
SBE (200 mg/day)	19.1±3.8	19.6±4.5	19.6±4.6		SBE (200 mg/day)	3.9±0.3	4.2±0.4††	4.2±0.4††	
SBE (400 mg/day)	18.6±3.8	21.1±4.8†	20.4±4.0		SBE (400 mg/day)	3.9±0.2	4.0±0.2†	4.1±0.3†	
ALT (U/L)					Cl (mEq/L)				
Placebo	19.3±8.8	18.5±7.7	19.1±9.4	5-45	Placebo	102±2	102±2	103±1†	98-108
SBE (200 mg/day)	18.3±7.2	19.8±8.5	18.6±10.6		SBE (200 mg/day)	102±2	102±3	102±2	
SBE (400 mg/day)	13.2±3.3	15.8±6.3†	14.8±3.9		SBE (400 mg/day)	102±2	103±2	102±1	
γ-GTP (U/L)					Ca (mg/dL)				
Placebo	32.1±19.9	31.6±19.8	31.1±16.9	Male <75	Placebo	9.5±0.3	9.4±0.4	9.3±0.3†	8.4-10.4
SBE (200 mg/day)	26.7±11.2	26.8±13.0	30.8±17.7	Female <45	SBE (200 mg/day)	9.4±0.4	9.5±0.3	9.4±0.4	
SBE (400 mg/day)	16.9±5.8	18.4±7.7	19.1±6.5†		SBE (400 mg/day)	9.5±0.3	9.4±0.4	9.3±0.2	
Amylase (U/L)					Fe (μg/dL)				
Placebo	77.5±25.6	79.4±22.5	82.3±26.6	40-122	Placebo	113±36	90±34	84±27†	Male 50-200
SBE (200 mg/day)	71.5±21.7	73.9±21.8	76.4±27.0†		SBE (200 mg/day)	113±36	105±38	103±29	Female 40-180
SBE (400 mg/day)	71.7±15.8	75.0±19.5	76.6±18.2†		SBE (400 mg/day)	113±35	102±36	103±36	
LDH (U/L)					IP (mg/dL)				
Placebo	181±18	180±23	180±18	120-240	Placebo	3.3±0.5	3.2±0.5	3.0±0.4†	2.5-4.5
SBE (200 mg/day)	179±22	181±22	179±21		SBE (200 mg/day)	3.5±0.5	3.2±0.5†	3.2±0.4†	
SBE (400 mg/day)	185±25	187±25	193±28		SBE (400 mg/day)	3.6±0.5	3.3±0.4††	3.3±0.6††	

Data are represented as the mean ±SD (n=16). Daggers denote significant differences vs. before ingestion at †: $p<0.05$ and ††: $p<0.01$.

Table 10. Changes of urine parameters

	Week	Placebo	SBE (200 mg)	SBE (400 mg)	Standard value
Protein	0	(-):14, (±):2	(-):14, (±):1, (+):1	(-):14, (±):2	(-)
	4	(-):14, (±):2	(-):15, (±):1	(-):15, (±):1	
	8	(-):14, (±):2	(-):14, (±):2	(-):16	
Glucose	0	(-):16	(-):16	(-):16	(-)
	4	(-):16	(-):16	(-):16	
	8	(-):16	(-):16	(-):16	
Urobilinogen	0	(±):16	(±):16	(±):16	(±)
	4	(±):16	(±):16	(±):16	
	8	(±):16	(±):16	(±):16	
Bilirubin	0	(-):16	(-):16	(-):16	(-)
	4	(-):16	(-):16	(-):16	
	8	(-):16	(-):16	(-):16	
pH	0	5.0:0 5.5:4 6.0:7 6.5:1 7.0:2 7.5:2	5.0:0 5.5:6 6.0:4 6.5:1 7.0:2 7.5:3	5.0:1 5.5:6 6.0:5 6.5:2 7.0:0 7.5:2	5.5-7.5
	4	5.0:0 5.5:6 6.0:4 6.5:3 7.0:3 7.5:0 8.0:0	5.0:0 5.5:4 6.0:5 6.5:2 7.0:2 7.5:2 8.0:1	5.0:1 5.5:1 6.0:6 6.5:4 7.0:2 7.5:2 8.0:0	
	8	5.5:7 6.0:2 6.5:2 7.0:3 7.5:1 8.0:1	5.5:6 6.0:4 6.5:3 7.0:1 7.5:2 8.0:0	5.5:2 6.0:3 6.5:6 7.0:2 7.5:3 8.0:0	
Occult blood	0	(-):16 (±):0 (+):0 (++):0 (+++):0	(-):14 (±):0 (+):1 (++):0 (+++):1	(-):13 (±):1 (+):2 (++):0 (+++):0	(-)
	4	(-):12 (±):1 (+):2 (++):1 (+++):0	(-):14 (±):1 (+):0 (++):0 (+++):1	(-):12 (±):1 (+):1 (++):1 (+++):1	
	8	(-):11 (±):2 (+):3 (++):0 (+++):0	(-):14 (±):2 (+):0 (++):0 (+++):0	(-):10 (±):2 (+):3 (++):1 (+++):0	
Ketone	0	(-):16	(-):15, (+):1	(-):16	(-)
bodies	4	(-):15, (+):1	(-):16	(-):15, (+):1	
	8	(-):16	(-):16	(-):16	

The number of subjects with each result is shown.

DISCUSSION

Seaberries are known to contain fatty acids, vitamins, polyphenols, and minerals [22]. Therefore, these berries have been used for nourishment and for treatment of various diseases, including gastrointestinal, skin, and liver conditions [23]. However, the potential health benefits of seaberry ingredients for urinary dysfunction have received little attention. Our previous *in vitro* study using rat bladder specimens and bladder cells demonstrated that triterpenoids derived from seaberries, including ursolic acid, suppressed excessive bladder contraction [19].

Overactive bladder (OAB) is an age-related urinary dysfunction syndrome that is especially frequent in women [24]. It may be related to neurologic dysfunction in addition to changes of the bladder musculature [25]. Recent research has shown that OAB symptoms are more severe in women with a high body mass index (BMI) than in those with a normal BMI [26]. In patients with diabetes, high glycosylated hemoglobin levels were also reported to be correlated with OAB [27]. Several questionnaires have been developed for diagnosis and assessment of OAB, including the Beck anxiety inventory (BAI) [28], OABSS [29], total urgency and frequency score (TUFS) [30], and actionable bladder symptom screening tool (ABSST) [31]. In the present study, the KHQ score and OABSS were employed as outcome measures. We found that intake of SBE (400 mg/day) for 8 weeks significantly improved the "Emotions" score in the KHQ, which is calculated from questions related to anxiety about urination. Among the KHQ items, questions 6a ("Does your bladder problem make you depressed?") and 6c ("Does your bladder problem make you feel bad about yourself?") also showed improvement after intake of SBE. Thereby, SBE was confirmed to improve KHQ scores, although the effect was mild. Intake of SBE (400 mg/day) for 8 weeks also improved the OABSS score for "How often do you have a sudden desire to urinate, which is difficult to defer?" According to Nishimura et al. [7], ingestion of pumpkin seed oil (10 g/day) for 6 to 12 weeks led to improvement of several items related to urgency and frequency of urination in the OABSS. Compared to the effects of pumpkin seed oil they reported, SBE suppressed OAB symptoms at a lower dosage in the present study. Additionally, there were no changes of the blood pressure, laboratory data, and urine parameters after intake of SBE for 8 weeks.

The present study had some limitations. First, this study was performed in healthy subjects who were not on medications for OAB, so the efficacy of SBE for subjects with a high OABSS or KHQ score was not clarified. Second, objective parameters of urinary function were not examined such as the intravesical pressure or residual urine volume. As the mechanism of SBE regarding the suppressive effect, we speculate the involvement of TGF-β. Several clinical biomarkers have been reported to change in urinary dysfunction. Urine TGF-β elevates in dysfunction of upper urinary tract [32] and is involved in hypertrophy of bladder muscle cells [33] and bladder inflammation [34]. Ursolic acid in SBE is an antagonist of TGF-β1 [35] and

we confirmed that ursolic acid suppressed gel contraction containing bladder smooth muscle cells induced by TGF-β1 [19]. Accordingly, suppression of TGF-β action by ursolic acid may be involved in the suppressive mechanism of SBE. However, it is unclear whether the response to SBE was actually based on improvement of OAB or due to an indirect influence on mood. Therefore, further investigation will be required to assess the objective effect of SBE on urinary function.

In conclusion, oral intake of SBE at 400 mg/day for 8 weeks reduced anxiety related to urination with no adverse effects. This finding suggests that SBE may be a safe and effective plant-derived food ingredient for improving symptoms of OAB.

CONCLUSION

We demonstrated that intake of seaberry extract (400 mg/day for 8 weeks) decreased anxiety related to urination. Therefore, seaberry extract may be useful for controlling symptoms of urinary dysfunction in persons with OAB.

List of Abbreviations: ABSST, actionable bladder symptom screening tool; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BAI, Beck anxiety inventory; BMI, body mass index; CK, creatinine kinase; GTP, glutamyltransferase; HDL, high density lipoprotein; IP, inorganic phosphorus; KHQ, King's Health Questionnaire; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; OAB, overactive bladder; OABSS, overactive bladder syndrome score; SBE, seaberry extract; TG, triglyceride; TUFS, total urgency and frequency score.

Competing Interests: The authors declare no conflicts of interest associated with this manuscript.

Authors' Contributions: Dr. Takara conducted the study and performed the tests. Dr. Shimoda prepared test samples and wrote the manuscript. Drs. Yamamoto and Suzuki coordinated the study and analyzed the data.

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