Quality Evaluation of Medicinal Products and Health Foods Containing Chaste Berry (*Vitex agnus-castus*) in Japanese, European and American Markets

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The aim of present study was to evaluate the qualities of chaste berry (fruit of *Vitex agnus-castus* L.) preparations using HPLC fingerprint analysis. Seven medicinal products 1 from Japan and 6 from Europe, and 17 health foods, 6 from Japan and 11 from the United States were analyzed. HPLC profile and 26 authentic peaks were compared medicinal products and health foods. Whereas medicinal products had similar HPLC profiles, health foods had various profiles and each peak was also greatly different. The measured amounts of two markers in 5 traditional medicinal products, agnuside and casticin specified in the European Pharmacopoeia (EP), the U.S. Pharmacopoeia (USP) or the WHO monographs of chaste berry, were much lower than those in 2 medicinal products defined as “well-established use” by the European Medicines Agency. The amounts of two markers for 17 health foods differed in a great deal from 14–5054% and 3–1272%, respectively. Furthermore the amount ratios of two markers, agnuside/casticin, in about half of the health foods were remarkably larger than the standard crude drug and the ratios were closer to one of the related Chinese herbs, *Vitex negundo* L. It is concluded that a combination of HPLC fingerprints and the amount ratios of the marker compounds of chaste berry preparations serves as a useful tool to evaluate the qualities of these preparations.

Key words chaste berry extract; *Vitex agnus-castus*; quality evaluation; HPLC fingerprint; health food

Chaste tree (*Vitex agnus-cactus* L., Family Verbenaceae) is a deciduous shrub or small tree native to Mediterranean Europe, central Asia and parts of India. Chaste berry, the fruit of chaste tree has been used for centuries for a variety of gynecologic conditions such as premenstrual syndrome (PMS). The exact prevalence of PMS is not known for certain, but estimates are that 70–90% of menstruating women have some degrees of physical or psychological symptoms before menses.¹⁾ Recently, randomized, placebo-controlled, double-blind clinical trials have clearly established the efficacy and safety of medicinal products containing chaste berry extracts.^{2–4)} These medical products have been successfully used for treating PMS throughout the European countries.

A great variety of medicinal products and health foods containing various kinds of herbal extracts have been distributed all over the world. A lot of health foods containing herbal extracts that are being used for medicine in Europe as well as imported American health foods are also currently in the Japanese market. It should be noted, however, that some of these foods may produce unexpected adverse effects,⁵⁾ and they often contain materials from different source, excess or lack of the materials, or a combination of both.^{6–13)} Being aware of this problem, a new medicinal product has been developed containing dry extracts of chaste berry in Japan. The aim of this study was to compare the qualities of medicinal products and health foods, by analyzing HPLC fingerprint profiles and

evaluating the contents of indicator constituents.

Experimental

Materials Seven medicinal products and seventeen health foods were used for test samples (Table 1). The medicinal product A has been developed in Japan, and six medicinal products B to G are marketed by major pharmaceutical companies in Europe. The six health foods H to M were purchased from a major online shopping site in Japan¹⁴⁾ and the eleven health foods N to X were purchased from the biggest online shopping site of the American botanical products.¹⁵⁾ All test samples were purchased from October 2009 to October 2011 except the medicinal product developed in Japan. As reference standard compounds, hydrophilic *p*-hydroxybenzoic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), amphiphilic isoorientin (Wako Pure Chemical Industries, Ltd.), agnuside (Phytoflan Diehm & Neuberger, Heidelberg, Germany) and lipophilic casticin (Phytoflan Diehm & Neuberger) were used. The crude drug of chaste berry (American Herbal Pharmacopoeia, Scotts Valley, U.S.A.) as the dried whole fruit of chaste tree (*Vitex agnus-castus* L.) was selected for the botanical standard crude drug. The fruit of *Vitex trifolia* L. and *Vitex rotundifolia* L. (Japanese standards for non-Pharmacopoeial crude drugs; Non-JPS), the fruit of *Vitex negundo* L. (unidentified in any pharmacopoeias) were purchased from Matsuura Yakugyo (Nagoya, Japan). The leaf of *Vitex negundo* L. (Chinese Pharmacopoeia; CP) was purchased in Chinese market. All specimens are deposited at Zeria Pharmaceutical Co., Ltd., Japan.

Preparation of Standard and Test Solutions Each reference standard compound was accurately weighed (5 mg)

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Table 1. Medicinal Products and Health Foods Containing Chaste Berry Used in This Study

Sample	Category	Area	Daily dosage and form ^{a)}	Crude drug equivalent (mg/d) ^{b)}	Indicated characterization
A	Medicine	Japan	20 mg dry extract in 1 tablet	180	9:1 extract
B	Medicine	Europe	20 mg dry extract in 1 tablet	180	6–12:1 extract (60% ethanol)
C	Medicine	Europe	12 mg dry extract in 1 tablet	201	15–18.5:1 extract (50% ethanol)
D	Medicine	Europe	4 mg dry extract in 1 tablet	36	7–11:1 extract (70% ethanol)
E	Medicine	Europe	4 mg dry extract in 1 tablet	40	7–13:1 extract (60% ethanol)
F	Medicine	Europe	4 mg dry extract in 1 capsule	40	7–13:1 extract (60% ethanol)
G	Medicine	Europe	3 mg dry extract in 1 tablet	39	10–16:1 extract (60% ethanol)
H	Food	Japan	7080 mg ^{c)} (150 drops)		
I	Food	Japan	175 mg powdered extract in 1 capsule		
J ^{d)}	Food	Japan	40 mg crude drug in 4 pellets	40	
K	Food	Japan	20 mg extract in 4 pellets		
L ^{d)}	Food	Japan	4.8 mg extract in 4 pellets		
M ^{d)}	Food	Japan	4.8 mg extract in 4 pellets		
N	Food	U.S.	6000 mg extract in 6 mL	6000	1:1 extract
O	Food	U.S.	1200 mg crude drug in 3 capsules	1200	
P ^{d)}	Food	U.S.	Extract and crude drug in 2 pellets ^{e)}	>750 ^{e)}	1.25 mg agnuside in 2 pellets
Q ^{d)}	Food	U.S.	680 mg extract in 2 capsules ^{f)}	500	
R	Food	U.S.	600 mg dry extract in 3 capsules		5% flavonoids in extract
S	Food	U.S.	500 mg extract in 2 capsules	900	0.6% agnuside in extract
T	Food	U.S.	450 mg dry extract in 2 capsules		0.5% agnuside in extract
U	Food	U.S.	240 mg powdered extract in 3 capsules		
V ^{d)}	Food	U.S.	Extract and crude drug in 1 capsule ^{g)}	>100 ^{g)}	0.6% agnuside in extract
W	Food	U.S.	225 mg extract in 1 capsule		0.6% agnuside in extract
X ^{d)}	Food	U.S.	200 mg extract in 2 pellets	2000	10:1 extract

a) In the case of the health foods (H–X), the recommended maximum daily intakes in the labels were listed. b) The crude drug equivalents in the extracts of European medicinal products (B–G) were calculated using the average of the drug-extraction ratios (DER) indicated in the patient information leaflets. The crude drug amounts (J, O) and the crude drug equivalent in the extracts (N–Q, S, X) indicated in the label of health foods were listed. c) Measured value. d) Combination preparations with other ingredients. e) Contain 250 mg extract and 750 mg crude drug in 2 pellets. f) Other ingredients were included in the extract. g) Contain 225 mg extract and 100 mg crude drug in 1 capsule.

and dissolved together with 80% methanol in the same 5 mL volumetric flask. Two milliliters of this solution was diluted to 50 mL with 80% methanol as the standard solution. Powdered crude drug (180 mg), powdered tablets and the contents in the capsules of the medicinal products (daily dosages) and of the health foods (recommended maximum daily intakes) were accurately weighed and mixed with 5 mL of 80% methanol. After vigorous shaking, the samples were centrifuged at 3000 rpm for 5 min. The supernatants were further filtered through a 0.45 μ m membrane filter (polyvinylidene difluoride (PVDF)) and used for the test solutions.

HPLC Conditions HPLC system consisted of separation module 2695, photodiode array detector 2998, and Empower 2 of the data processing equipment (Waters, Milford, MA, U.S.A.). The chromatographic separation was carried out on symmetry C₁₈ column (100 mm $\times$ 4.6 mm i.d., Waters) maintained at 35°C. The mobile phases were methanol (a) and 0.5% phosphoric acid (b) with a gradient program as follows: a/b=5/95 (0 min) $\rightarrow$ 10/90 (3 min) $\rightarrow$ 15/85 (7 min) $\rightarrow$ 25/75 (19 min) $\rightarrow$ 27/73 (25 min) $\rightarrow$ 30/70 (30 min) $\rightarrow$ 34/66 (39 min) $\rightarrow$ 37/63 (44 min) $\rightarrow$ 50/50 (49 min) $\rightarrow$ 90/10 (75 min) and held 5 min of 100% methanol at flow rate of 1.0 mL/min. The detector collected all spectral information between 210 nm and 450 nm and chromatographic peaks were monitored at 275 nm. The injection volume was 10 μ L.

Identification of Peak Spectra Obtained from HPLC Each peak area was detected by the automatic integration method. The typical 26 peaks obtained from the standard crude drug were selected as standardized peaks. Those peaks

corresponded to about 80% of the all peak areas obtained from the standard crude drug. Identification of each peak obtained from test samples without seven health foods of combination preparations with other ingredients was performed as follows: each peak from the test samples was identified by comparing the UV spectra with those of the 26 standardized peaks. The quantitation limits for this analysis are approximately 4 ng (0.4 μ g/mL) of *p*-hydroxybenzoic acid and 2 ng (0.2 μ g/mL) of isoorientin, agnuside or casticin.

Quantitative Determination of Agnuside and Casticin Contents of agnuside and casticin in the preparations were calculated as follows: contents (mg/day) = W_s (mg) $\times A_T/A_S \times DF$, where A_T and A_S are the peak areas of agnuside or casticin in test solution and standard solution, respectively, W_s is the weight of agnuside or casticin and DF is the dilution factor (0.04). The calibration curves were exhibited excellent linear regressions of $r^2=1.0000$, over the concentration range of 0.24–1000 μ g/mL for agnuside and 0.1–500 μ g/mL for casticin, respectively.

Results and Discussion

HPLC Fingerprints HPLC chromatograms of marker compounds (*p*-hydroxybenzoic acid, isoorientin, agnuside and casticin), a botanical standard crude drug of chaste berry and representative test samples (the medicinal products, A, C, D, and the health foods, K, N) are shown in Fig. 1. HPLC chromatograms of representative test samples (Fig. 1c) revealed to be high similar to that of the standard crude drug (Fig. 1b). Peak area percentages of HPLC chromatograms in seven

Fig. 1. HPLC Fingerprint Chromatograms of (a) Marker Compounds, (b) Standard Crude Drug of Chaste Berry (Fruit of *Vitex agnus-castus* L.) and (c) Representative Test Samples^{a)}

a) Three medicinal products in three different concentrations of extraction solvents, A: 60%, C: 50% and D: 70% ethanol, and two health foods in different amounts of chaste berry extracts without combination preparations, K: 20mg and N: 6000mg. Absorbance unit of health food N was reduced by one third.

medicinal products and ten health foods without combination preparations were analyzed and their profiles were compared (Table 2).

The medicinal products A and B showed HPLC profiles similar to that of the standard crude drug with all 26 peaks. Two to four out of 26 peaks were not detected in other medicinal products. All missing peaks corresponded to those of minor constituents which in the standard crude drug were less than 2%. Other than those minor differences, the HPLC profiles of seven medicinal products were similar to that of the standard crude drug.

On the other hand, much more heterogeneity in the number of peaks was seen in health foods (Table 2). Two health foods H and K (Table 1) containing chaste berry extracts as being equal to or surpassing the level of amounts with the medicinal products were without 6 and 10 peaks, respectively. In addition, peak 6, 8, 15 and 22 detected in all medicinal products and the standard crude drug were not detected in six health foods. There were no health foods with all 26 peaks obtained from the standard crude drug. These data indicate that some of these health foods may have been prepared utilizing differ-

ent crude drugs and/or different manufacturing methods. It is likely that some of these health foods may produce different pharmacological effects.

Amounts of Marker Compounds To further define the quality of each medicinal product or health food, the major constituents of the crude drug for the preparations were examined. Hydrophilic iridoids, agnuside and hydrophobic flavonoids, casticin are under strict regulation as described in the European Pharmacopoeia (EP), the U.S. Pharmacopoeia (USP) or the WHO monographs¹⁶⁾: dried crude drug of chaste berry contains not less than 0.05% agnuside and 0.08% casticin by HPLC.

The Committee on the Herbal Medicinal Products (HMPC), a sub-organization of the European Medicines Agency, has been evaluating herbal medicines in Europe, and published a final report on chaste berry in 2010,^{17,18)} in which an oral preparation of chaste berry extracts for "Well-established use" are defined as follows: the medicinal preparations of chaste berry should contain the dry extract of the crude drug, which is extracted with 60% ethanol aqueous solution at the drug-extract-ratio (DER) of 6–12:1, equivalent to 180mg/d of the

Table 2. Peak Area Percentages of HPLC Chromatograms Obtained from Medicinal Products (A–G) and Health Foods (H–W)^{a)}

Peak number ^{b)}	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
Retention time (min) ^{c)}	5.7	8.9	9.4	11.5	11.7	17.8	20.7	24.0	25.2	25.8	26.4	30.8	32.6	33.0	35.2	39.1	40.9	42.4	52.6	55.3	56.0	56.2	58.6	61.7	63.4	63.9	
Crude drug ^{d)}	2.1	16.9	1.6	4.2	1.1	3.3	1.0	1.5	6.5	7.2	1.0	2.0	4.3	1.5	1.1	2.4	1.3	1.3	1.4	1.4	24.1	1.7	1.2	4.6	1.1	4.2	
A	3.7	23.9	2.2	5.4	1.6	2.7	0.1	2.6	10.1	7.8	0.9	2.6	4.3	0.8	0.7	0.1	1.5	2.7	0.3	0.6	17.3	2.0	0.2	3.5	0.4	1.8	
B	3.8	18.0	2.6	5.1	1.4	2.5	0.3	2.5	9.0	11.4	0.4	2.6	4.5	0.9	1.5	0.2	1.7	2.5	0.3	0.7	18.4	2.0	0.5	4.6	0.4	1.9	
C	4.6	26.7	1.4	3.4	1.3	4.0	—	1.1	6.2	2.3	0.1	1.4	1.6	—	0.3	0.2	1.1	0.7	19.9	0.9	15.4	1.7	0.6	3.0	0.4	1.7	
D	2.4	9.5	2.2	6.0	0.7	2.1	0.4	1.6	14.1	23.8	0.6	3.1	2.8	5.8	3.1	—	—	—	—	0.6	0.9	15.1	1.5	0.3	2.6	—	0.8
E	3.2	24.7	1.5	6.5	1.2	3.3	—	1.2	11.1	11.1	—	2.4	3.0	—	0.7	—	2.2	0.7	0.5	0.8	17.3	1.7	0.8	2.8	0.5	2.7	
F	5.8	21.7	1.9	5.4	1.2	2.7	—	1.6	10.0	13.0	0.5	2.4	2.8	—	1.3	1.1	1.8	1.2	0.7	0.9	16.0	1.5	0.6	3.2	0.5	2.3	
G	4.4	15.7	1.3	5.2	1.3	2.8	—	1.8	8.4	15.9	0.2	1.8	4.4	0.8	2.1	—	1.7	1.0	0.6	0.9	18.9	1.9	1.0	3.4	0.8	3.8	
H	8.2	13.8	1.3	7.9	1.2	2.1	0.3	—	11.8	39.9	—	3.2	0.3	—	—	0.1	0.5	—	0.3	0.4	7.4	—	0.1	0.6	0.1	0.5	
I	10.5	34.6	2.3	4.4	5.3	0.8	0.4	0.5	0.5	6.1	0.1	0.7	0.3	—	—	0.2	—	0.3	4.8	0.1	27.2	0.1	0.1	0.1	0.1	0.6	
K	5.9	29.5	2.0	4.5	1.3	—	—	—	10.0	5.6	—	2.9	—	—	—	—	—	0.3	1.3	1.3	24.2	—	0.5	4.0	1.1	5.5	
N	7.6	61.0	1.9	6.6	4.3	0.2	0.1	—	0.9	6.1	0.4	1.7	1.4	—	0.1	0.2	0.5	0.1	0.2	0.2	3.9	1.0	0.02	0.5	0.2	0.9	
O	5.6	40.5	1.8	7.6	2.8	—	0.2	0.3	7.1	16.0	0.4	1.9	1.0	—	0.02	0.4	0.5	0.1	0.3	0.2	5.3	1.1	0.1	1.4	1.0	4.4	
R	10.2	32.6	2.5	4.7	5.5	—	0.5	0.5	3.6	7.5	0.1	0.6	0.3	0.02	0.1	0.4	1.4	0.3	4.7	0.3	22.2	0.1	0.1	0.5	0.3	1.0	
S	3.7	19.4	1.8	5.6	1.2	0.2	0.3	0.2	13.4	16.4	0.2	2.3	0.5	0.03	0.1	0.5	—	0.2	0.5	0.8	26.6	—	0.3	4.0	0.3	1.3	
T	8.6	33.4	2.0	13.2	—	0.1	0.3	0.2	8.7	18.9	—	3.6	0.6	0.04	—	0.5	—	0.1	0.4	0.2	3.9	0.8	0.1	0.7	0.7	3.1	
U	6.1	20.4	1.6	10.4	—	1.9	0.5	0.2	7.4	32.7	—	3.6	0.9	—	—	0.5	0.5	0.1	0.4	0.3	5.8	1.3	0.1	1.4	0.7	3.2	
W	4.9	24.6	0.4	1.6	0.5	1.9	0.6	0.1	10.4	38.8	0.1	4.9	0.8	—	—	0.5	0.6	0.1	0.4	0.3	4.4	0.9	0.04	1.0	0.4	1.7	

a) Peak area percentages in the Table were calculated from the peak area of each component to the sum of the 26 peak areas recorded in the chromatogram obtained from preparations without combination preparations of health foods containing other ingredients (J, L, M, P, Q, V and X); —: absence of the peak. b) Bold face types indicate reference standard compounds as follows: *p*-hydroxybenzoic acid (No. 2), isoorientin (No. 9), agnuside (No. 10) and casticin (No. 21). c) Obtained from a standard crude drug. d) A standard crude drug of chaste berry (fruit of *Vitex agnus-castus* L.) verified by the AHP.

Table 3. Measured Amounts of Agnuside and Casticin in the Medicinal Products and the Health Foods Used for Estimating the Amounts of These Two Markers in the Crude Drug as the Raw Material for the Preparation Concerned^{a)}

Sample	Category	Area	Crude drug equivalent ^{b)} (mg/d)	Measured amount in the preparations (mg/d)		Estimated amount in the crude drug (%) ^{c)}	
				Agnuside	Casticin	Agnuside	Casticin
A	Medicine	Japan	180	0.19	0.16	0.15	0.13
B	Medicine	Europe	180	0.25	0.16	0.20	0.13
C	Medicine	Europe	201	0.03	0.07	0.02	0.05
D	Medicine	Europe	36	0.11	0.03	0.44	0.12
E	Medicine	Europe	40	0.04	0.03	0.14	0.11
F	Medicine	Europe	40	0.20	0.05	0.71	0.18
G	Medicine	Europe	39	0.04	0.02	0.15	0.07
J	Food	Japan	40	0.013	0.004	0.03	0.01
N	Food	U.S.	6000	0.68	0.17	0.02	0.004
O	Food	U.S.	1200	1.95	0.25	0.16	0.02
Q	Food	U.S.	500	2.91	0.25	0.83	0.07
S	Food	U.S.	900	3.04	1.83	0.48	0.29
X	Food	U.S.	2000	0.77	0.02	0.06	0.001

a) All medicinal products and 6 health foods indicating the crude drug equivalents in the preparations were shown. b) The same as Table 1. c) Estimated amounts were calculated by use of the crude drug equivalents and the measured amounts based on the presumption that the extraction rates of agnuside and casticin from crude drug to the extract are 70% except for the health foods (J, O) containing only powdered crude drug instead of extracts. Bold face types indicate the out of specifications for the amounts of agnuside (0.05%<) or casticin (0.08%<) defined in the EP, the USP or the WHO Monographs.

crude herbal substances. All other medicinal products are defined as traditional products. According to the definition, two medicinal products A and B are defined as “Well-established use” and the other five medicinal products C to G are traditional products.

The specified contents of 0.05% agnuside and 0.08% casticin in the crude drug are considered to be the minimum requirement for each marker cited above. Accordingly, a medicinal product equivalent to 180mg/d of the crude drug recommended by HMPC should contain at least 0.09mg/d of agnuside and 0.144mg/d of casticin, respectively.

The two medicinal products A and B defined as “Well-established use” contained 246% of agnuside and 111% of casticin of minimum requirement on average. The five traditional medicinal products C to G contained less amounts of casticin as 14–51% and agnuside as 31–221% of minimum requirement. On the other hand, 17 health foods varied in a wide range from 14 to over 5054% for agnuside and 3 to 1272% for casticin, respectively. The Japanese health food J contained the least content of these two markers, 14% for agnuside and 3% for casticin. In contrast, some of the American health foods (P, S, T, V) contained larger amounts of either one or both markers, and thus caution should be exercised for possible untoward effects, even though chaste berry has been used for a long time and serious adverse effects have never been reported.^{19,20)}

Estimated Qualities of Crude Drugs Used for Chaste Berry Preparations We examined an extraction rate of 60% ethanol solution for agnuside and casticin using several samples of chaste berry crude drug and found that approximately 70% of these marker compounds in each crude drug were extracted (data not shown). With a fixed extraction rate at 70% and the measured data for the two markers of each extract preparation, the amounts of these markers in the corresponding crude drug used as the raw material for all medicinal products and 4 health foods (N, Q, S, X) can be estimated for further consideration (Table 3). To perform a similar estimation for two health foods (J, O) contained only powdered

Fig. 2. Measured Concentration Ratio of Agnuside-to-Casticin in the Crude Drug of 7 Medicinal Products and 17 Health Foods

crude drugs, the amounts of these markers in the crude drug can be readily obtained (Table 3).

The estimated amounts of the two markers in the crude drug for medicinal products other than C are well above the respective minimum standard with an exception for the amount of casticin of the medicine G. In contrast, the estimated amounts of the two markers for the medicine C were much lower than the respective minimum standard. The medicine C was supposed to be manufactured under the good manufacturing practice (GMP) guideline by a crude drug defined by the EP and 50% ethanol solution for the extraction defined by the Complete German Commission E Monographs.²¹⁾ The reason for the discrepancy is not known at present, however, some steps of manufacturing procedure may have been responsible.

In contrast to the medicinal products, the estimated amounts of the two markers were much lower than the minimum standard in most of the health foods: J and N contained much less amounts of both agnuside and casticin; O, Q and X were estimated to contain much lower amounts of casticin. This may have resulted from the use of crude drugs that do not meet the standard defined in EP, the USP or the WHO monographs or of improper extraction solvent such as water or

Table 4. Amounts of Agnuside and Casticin in Chaste Berry (Fruit of *Vitex agnus-castus* L.) and Related Crude Drugs

Botanical name	Part	Specification ^{a)}	Amount (%)		Agnuside/Casticin
			Agnuside	Casticin	
<i>Vitex agnus-castus</i> L.	Fruit	AHP	0.094	0.126	0.7
<i>Vitex trifolia</i> L.	Fruit	Non-JPS	0.082	0.029	2.8
<i>Vitex rotundifolia</i> L.	Fruit	Non-JPS	0.001	0.003	0.3
<i>Vitex negundo</i> L.	Leaf	CP	4.320	0.015	288.0
<i>Vitex negundo</i> L.	Fruit	Unidentified	0.019	0.0003	63.3

a) AHP; the American Herbal Pharmacopoeia, Non-JPS; the Japanese standards for non-Pharmacopoeial crude drugs, CP; the Chinese Pharmacopoeia.

a lower concentration of ethanol, indicating a possibility that either a crude drug incompatible to the official standards or a combination of crude drugs different from that described in the label are used.

As for the remaining 11 health foods (H, I, K, L and M from Japan, and P, Q, R, S, T and V from U.S.), the amounts of both markers could not be estimated due to the lack of information in their labels (Table 1).

Qualities of Chaste Berry Extracts in Health Foods The origin of crude drug for the medicinal products is exclusive to *Vitex agnus-castus* L. However, according to the American Herbal Pharmacopoeia (AHP) Monograph,²²⁾ the most commonly found adulterants of *Vitex agnus-castus* L. in trade are *Vitex trifolia* L., *Vitex rotundifolia* L. and *Vitex negundo* L. All four species are called chaste tree or chaste berry in the United States. Adulteration of *Vitex agnus-castus* L. occurs in trade rather than during collection since its geographic range does not overlap with that of the other species.^{22,23)} Adulteration may be one of the factors causing unexpectedly lower or higher content of agnuside and/or casticin in health foods (Table 3). To test this possibility, agnuside-to-casticin ratio in those health foods was calculated (Fig. 2). The ratio was 0.75 for the standard crude drug of chaste berry and varied between 0.4 and 4.1 for medicinal products. In contrast, the ratio was much higher in the health foods and reached 10-times or higher (Fig. 2). As Compared to *Vitex agnus-castus* L., the leaf and fruit of *Vitex negundo* L. (the ratio: 288.0 and 63.3, respectively) and the fruit of *Vitex trifolia* L. (2.8) revealed a particularly higher ratio as shown in Table 4.

In China, the major production area of these related plants, various chaste berry extracts labeled with product-in-China are on the market.²⁴⁾ We can easily find that various chaste berry extracts marketed in China are standardized by only agnuside, although the crude drug of chaste berry is standardized by both of agnuside and casticin in the USP and the WHO monographs. In addition, the American health foods P, S, T, V and W were labeled with amount of only agnuside. These data are likely to support the following conclusion: to elevate the ratio to the level higher than 10, it is necessary either to use the leaf or the fruit of *Vitex negundo* L. as the source or one of the sources of the crude drug preparation, or to add a significant amount of semi-purified agnuside to the original preparation that does not meet the standard crude drug of chaste berry, although the latter seems to be more difficult to practice. Whether accidental or intentional, something like this may have been happening in some of health foods in the U.S. market and industry.

Conclusion

This study clearly demonstrated a possibility that some health foods may have been manufactured using either crude drugs without conforming official standard, different amount of crude drugs, and/or chaste berry extracts prepared through unauthorized procedure, including putting intentionally some marker compounds to their products.

Although more refinement may be necessary, the method used in this study will provide a useful tool for quality evaluation of chest berry-containing medicinal products or health foods by comparing HPLC profiles and authentic peaks with standard crude drug and the contents as well as amount ratio of two indicator compounds.

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