



Evaluation of the crude drugs by means of colorimeter. Part 6.¹⁾ Correlation between the color and total polyphenol content of Geranium Herb

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The color of Geranium Herb, which is a quite popular folk medicine for intestinal disorders such as diarrhea, was investigated for the quality evaluation by means of colorimeter. The antidiarrheal effect of Geranium Herb is considered to be due to tannin, which is a kind of polyphenols. On various Geranium Herb, we examined the correlation between the total polyphenol content and color of both the powder and water extract treated with the iron (III) chloride TS. As the result, we found that Geranium Herb with a higher total polyphenol content tended to show lower color index L^* value in the water extract treated with the iron (III) chloride TS. In addition, the total polyphenol quantity was high, while L^* value was low in the foliar part. On the other hand, total polyphenol quantity was low, while L^* value was high in the stem part. Therefore, when the effect of total polyphenol is expected, Geranium Herb with a low ratio of stem part is thought to be of good quality, and it is considered that color index L^* value of the Geranium Herb water extract treated with the iron (III) chloride TS is available in the quality evaluation for Geranium Herb.

Key words Geranium Herb, quality evaluation, colorimeter, CIE 1976 $L^*a^*b^*$ color system.

Introduction

The crude drugs are produced widely in various regions and countries, and hence objective quality evaluation of crude drugs desired a uniform level quality of properties. Even today, we still depend on the five senses for quality evaluation of crude drugs, in particular color, odor and taste are very important. Since crude drugs contain many components within one product, it is difficult to evaluate its quality by component quantity assay or other standard method. In recent years, use of a colorimeter, which measures the color of an object and fluid, has become simple and easy. In this study, we investigated the color of Geranium Herb by using the colorimeter.

Geranium Herb, or “Gennoshoko” in Japanese is a quite popular folk medicine used for intestinal disorders such as diarrhea. Geranium Herb is sourced from the aerial part of *Geranium thunbergii* Siebold et Zuccarini (*Geraniaceae*) in the Japanese Pharmacopoeia (JP). It is generally said that the name of “Gennoshoko” came from the fact that the antidiarrheal effect which appeared immediately.²⁾ The antidiarrheal effect of Geranium Herb is considered to be due to tannin, which is a kind of polyphenols.³⁾ An identification test of tannin in Geranium Herb is prescribed in JP, however the quantitative analysis is not. Therefore, the quality of Geranium Herb in the market is likely to vary widely. In the present paper, we measured the color of the powder and the water extract after reaction with the iron (III) chloride TS. Then, the correlation between these color data and total polyphenol content were investigated.

Materials and Methods

Materials. Twelve Geranium Herb samples were given by Uchida Wakanyaku Co., Ltd. (Tokyo, Japan) and Tochimoto Tenkaido Co., Ltd. (Osaka, Japan).

Preparation of Geranium Herb samples: Geranium Herbs and as well as divided leaf parts and as stem parts were used for the experiments. They were powdered using an electric mill and passed through a 100-mesh sieve (150 μ m) to give fine to very fine powder.

Determination of the total polyphenol. Folin-Ciocalteu method: The total polyphenol content was measured using Folin-Ciocalteu method.⁴⁾ In brief, 1.0 mL of sample solution and 2-fold diluted Folin-Ciocalteu reagent (Wako Pure Chemical Industries, Ltd. Osaka, Japan) were mixed in the tube. After 3 min at room temperature, 5 mL of 0.4 M Na_2CO_3 (Wako Pure Chemical Industries, Ltd.) was added. The tubes were mixed thoroughly, kept for over 2 hours at room temperature, and read at 765 nm using gallic acid as a standard, with the results expressed as gallic acid equivalents.

Preparation of sample solution: 100 mg of the sieved sample was weighed out accurately, added to 10 mL of 80% methanol (Wako Pure Chemical Industries, Ltd.) and sonicated for 10 min. After centrifuging at $1,710 \times g$ for 10 min, supernatant was collected to give the sample solution for Folin-Ciocalteu method.

Standard curve: 200 mg/L 80% methanol solution of gallic acid monohydrate (Wako Pure Chemical Industries, Ltd.) was serially diluted to make gallic acid standard solutions of 20, 40, 50, 80, 100, 150 mg/L. They were then

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analyzed by Folin-Ciocalteu method to get the standard curve. The correlation coefficient of the standard curve was $r^2 = 0.99$.

Measurement of color by colorimeter. Instrument: Colorimeter (Konica Minolta Holdings, Inc. Tokyo, Japan), CR-200 was used for the measurement of the sieved powder samples and CT-210 used for the measurement of water extract treated with the iron (III) chloride TS. CIE 1976 $L^*a^*b^*$ color system were used as reported before.⁵⁾ In the $L^*a^*b^*$ color system, brightness is expressed in L^* (0 - 100, dark to bright), and chromaticity to show a hue and chroma is expressed in a^* and b^* values. The high value of L^* shows that a color of a measured object is bright. While, a^* and b^* show a chromatic direction in which $+a^*$ shows a red direction, $-a^*$ shows a green direction, $+b^*$ shows a yellow direction and $-b^*$ shows a blue direction, respectively. These values show large measurement to a brightly-colored object.

Water extract of Geranium Herb samples: 50 mg of the sieved sample was weighed out accurately and added to 40 mL of ultrapure water, and extracted in the boiled water bath for 20 min. The extract was filtrated and adjusted to exactly 50 mL with ultrapure water. 10 mL of adjusted solution was added to 1 mL of the iron (III) chloride TS.

Statistics. Data were represented as the mean \pm S.D. of twelve samples of Geranium Herb. Statistical significance was determined by non-paired Student's *t*-test.

Result and Discussion

It is considered that the tannins, mainly those in the leaves⁶⁾ of Geranium Herb, show an antidiarrhea effect.³⁾ In this study, we tested twelve samples of Geranium Herb in the market produced in 1999 ~ 2005. The total polyphenol content was significantly higher in the leaf part of them than in the stem part (Fig. 1, Table 1), the same result as reported by Okuda *et al.*⁷⁾ A comparison of the color of the powder from the leaf part against that from the stem part showed significant differences in each color indices L^* , a^* and b^* (Fig. 2, Table 2). Meanwhile, water extract treated with the iron (III) chloride TS also showed significant differences in L^* and a^* , but not in b^* (Fig. 3, Table 2). Looking at the ratio of leaf part in twelve samples of Geranium Herb, there is a range from 11.81 to 34.83 % (Table 1). The good quality of Geranium Herb is considered when including a lot of leaves.⁶⁾ During the process of collecting and carrying Geranium Herb, it seems that some leaves get lost before the product appears in the market. However, most of the circulating market articles of Geranium Herb are in cut form, thus, it is difficult to guess a foliar ratio in them. We found the correlation relatively between total polyphenol quantity in Geranium Herb and a ratio of leaf in it ($r^2 = 0.40$) (Fig. 4). However, no correlation was found between total polyphenol quantity in Geranium Herb and any color indices of the powdered Geranium Herb (Fig. 5). In contrast, a correlation was found between total polyphenol quantity in Geranium Herb and a color index L^* of the water extract treated with the iron (III) chloride TS ($r^2 = 0.62$)

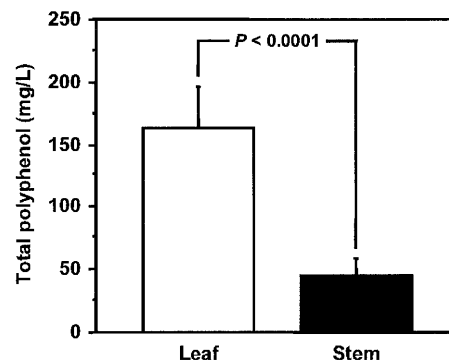


Fig. 1 Total polyphenol content of Geranium Herb samples. Open bar shows the leaf part and closed bar shows the stem part, respectively. Value represents mean \pm S.D. of twelve samples of Geranium Herb.

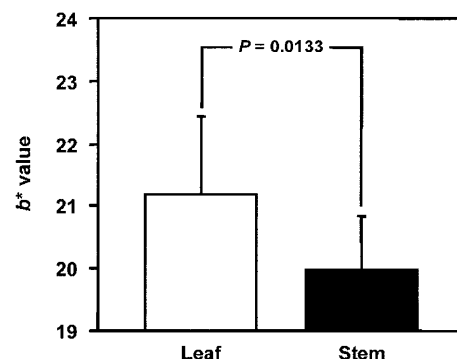
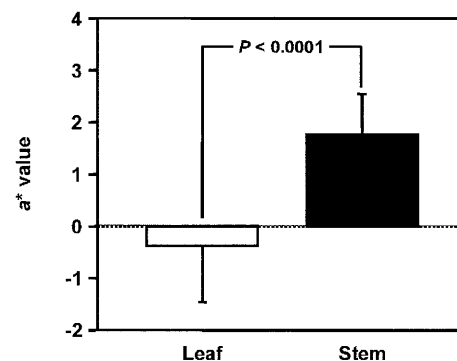
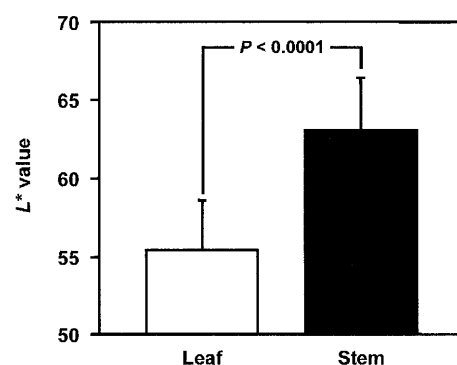


Fig. 2 Color indices of powdered Geranium Herb samples. Open bar shows the leaf part and closed bar shows the stem part, respectively. Each value represents mean \pm S.D. of twelve samples of Geranium Herb.

Table 1 Total polyphenol content in Geranium Herb samples.

Sample No.	Place of Original	Year	GL*/GH* (%)	Total Polyphenol content in GL* (mg/L)**	Total Polyphenol content in GS* (mg/L)**	Total Polyphenol content in GH* (mg/L)**
1	Niigata	1999	11.81	169.59	56.24	64.04
2	Nagano	1999	34.83	175.59	38.80	99.32
3	Nagano	2000	27.74	173.52	40.32	90.67
4	Nagano	2001	32.51	183.71	47.90	101.22
5	Niigata	2002	21.36	61.94	13.25	41.68
6	Nagano	2002	31.17	167.23	42.70	102.11
7	Nagano	2003	23.57	163.33	53.44	84.10
8	Tokushima	2004	21.06	184.81	62.92	99.85
9	Nagano	2004	29.19	162.79	48.44	83.24
10	Niigata	2005	13.84	163.76	39.22	64.64
11	Japan	2005	31.49	165.63	52.37	87.97
12	China	2005	26.57	190.08	52.26	113.43

*Abbreviation; GH: Geranium Herb, GL: Leaf part of GH, GS: Stem part of GH

**As gallic acid equivalents

Table 2 Color indices of powdered Geranium Herb samples and water extract treated with the iron (III) chloride TS.

Sample No.		Powder			Water extract treated with the iron (III) chloride TS		
		<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>L</i> *	<i>a</i> *	<i>b</i> *
Geranium Herb (GH)	1	67.46	1.90	18.98	84.32	-4.02	34.68
	2	53.93	1.60	20.09	68.25	-1.30	37.03
	3	57.24	0.50	21.91	72.34	-1.99	36.92
	4	59.71	-0.12	21.71	73.14	-2.32	36.46
	5	56.85	1.26	19.77	81.33	-2.63	36.93
	6	58.82	-0.09	22.15	71.35	-1.92	37.07
	7	57.68	0.80	19.74	73.57	-1.89	36.76
	8	60.79	-0.73	20.33	77.00	-2.69	35.74
	9	58.74	0.29	20.74	76.41	-2.52	36.37
	10	63.71	-0.61	20.45	80.91	-3.46	36.20
	11	59.70	-0.79	20.49	76.56	-2.46	36.34
	12	60.10	-0.30	20.95	73.17	-2.48	36.23
	Ave.	59.56	0.31	20.61	75.70	-2.47	36.39
	S.D.	3.45	0.92	0.95	4.66	0.72	0.67
Leaf part of Geranium Herb (GL)	1	62.66	-1.32	21.29	60.71	-3.75	30.55
	2	50.53	1.35	20.31	55.43	-1.45	31.99
	3	52.51	0.55	21.52	55.71	-1.58	31.77
	4	54.26	-0.08	22.29	54.92	-1.93	31.26
	5	52.18	1.19	18.09	73.43	-1.91	35.74
	6	54.67	0.29	21.55	56.13	-1.53	32.05
	7	54.07	0.19	20.78	58.62	-1.62	32.46
	8	56.67	-1.24	20.96	60.99	-2.36	31.50
	9	55.31	-0.59	21.95	60.93	-2.13	32.27
	10	58.34	-1.23	20.15	63.17	-2.80	32.62
	11	55.58	-1.93	23.00	60.36	-1.00	35.34
	12	57.57	-1.55	22.20	56.31	-1.25	34.76
	Ave.	55.36	-0.36	21.17	59.73	-1.94	32.69
	S.D.	3.22	1.10	1.28	5.11	0.75	1.67
Stem part of Geranium Herb (GS)	1	70.41	2.36	19.49	86.10	-4.83	32.73
	2	57.66	3.06	20.57	82.39	-3.37	33.16
	3	62.03	1.36	20.81	84.42	-3.95	32.38
	4	63.57	1.32	20.65	85.59	-4.13	32.22
	5	58.60	3.05	20.14	90.82	-4.42	30.85
	6	63.16	1.46	20.97	85.07	-4.01	32.06
	7	61.04	1.94	20.32	83.43	-3.65	32.47
	8	64.84	1.84	19.12	86.44	-3.74	32.69
	9	62.65	1.55	19.69	85.06	-3.87	32.22
	10	65.00	0.61	20.77	86.41	-4.44	32.11
	11	63.93	0.84	18.27	87.72	-4.16	34.41
	12	64.34	1.76	18.76	85.72	-3.95	35.37
	Ave.	63.10	1.76	19.96	85.76	-4.04	32.72
	S.D.	3.27	0.76	0.89	2.13	0.39	1.17

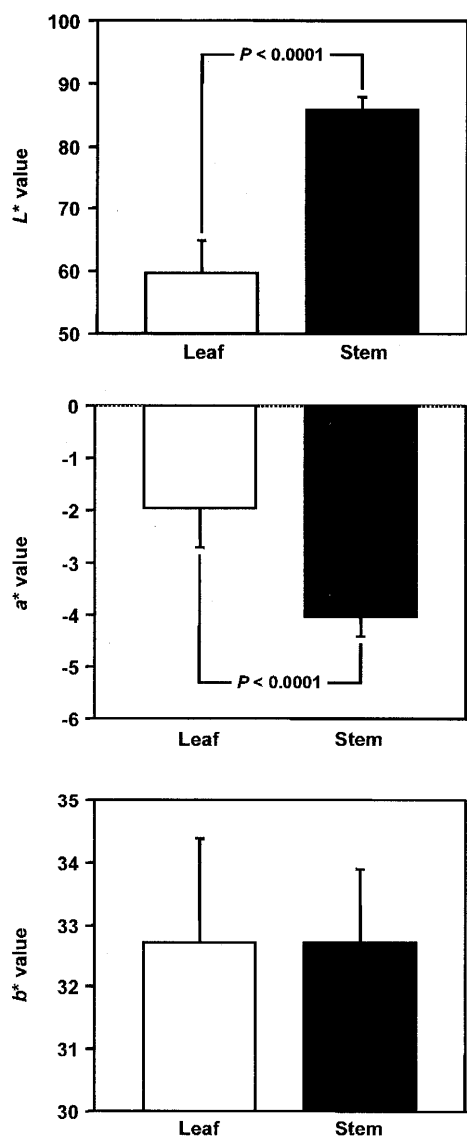


Fig. 3 Color indices of water extract treated with the iron (III) chloride TS of Geranium Herb samples. Open bar shows the leaf part and closed bar shows the stem part, respectively. Each value represents mean \pm S.D. of twelve samples of Geranium Herb.

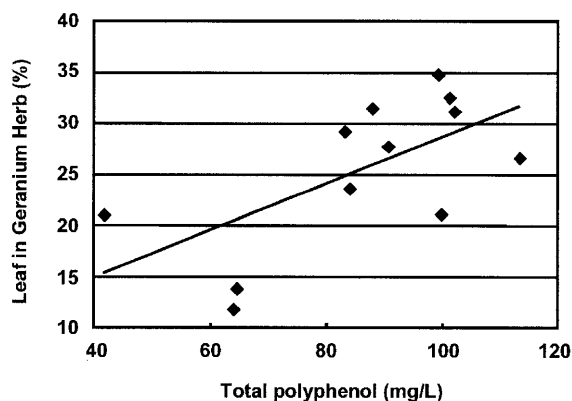


Fig. 4 Correlation between total polyphenol content and a ratio of leaf in Geranium Herb. Correlative coefficient is 0.40.

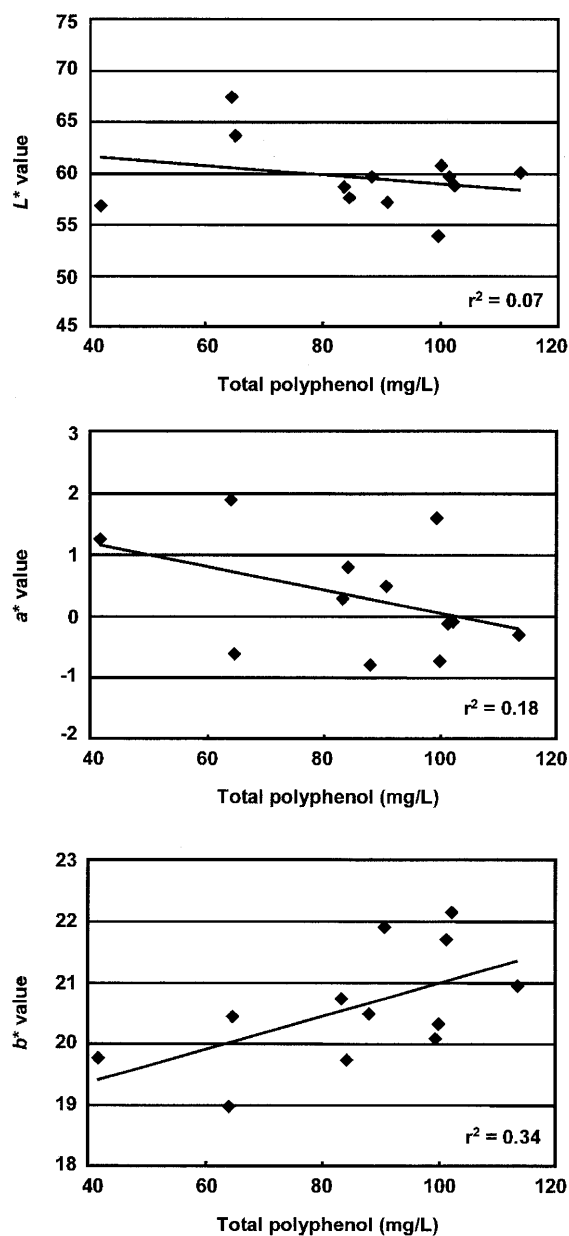


Fig. 5 Correlation between total polyphenol content in Geranium Herb and color indices of powdered Geranium Herb. Correlative coefficients are shown in each graph.

(Fig. 6).

In this study, we used samples collected each summer over seven years. We found no relation in the total polyphenol quantity between old samples and new ones. Therefore, it is thought that there is no relation in the production year in particular when Geranium Herb is used in anticipation of constipating action. It is expected that Geranium Herb having bright color and good scent is liked better when it is used like tea. The color index $-a^*$ indicates the grade of green, which didn't show the correlation with total polyphenol quantity of the Geranium Herb in this study. This result suggests the necessity for study on correlation between color indices and the other organoleptic

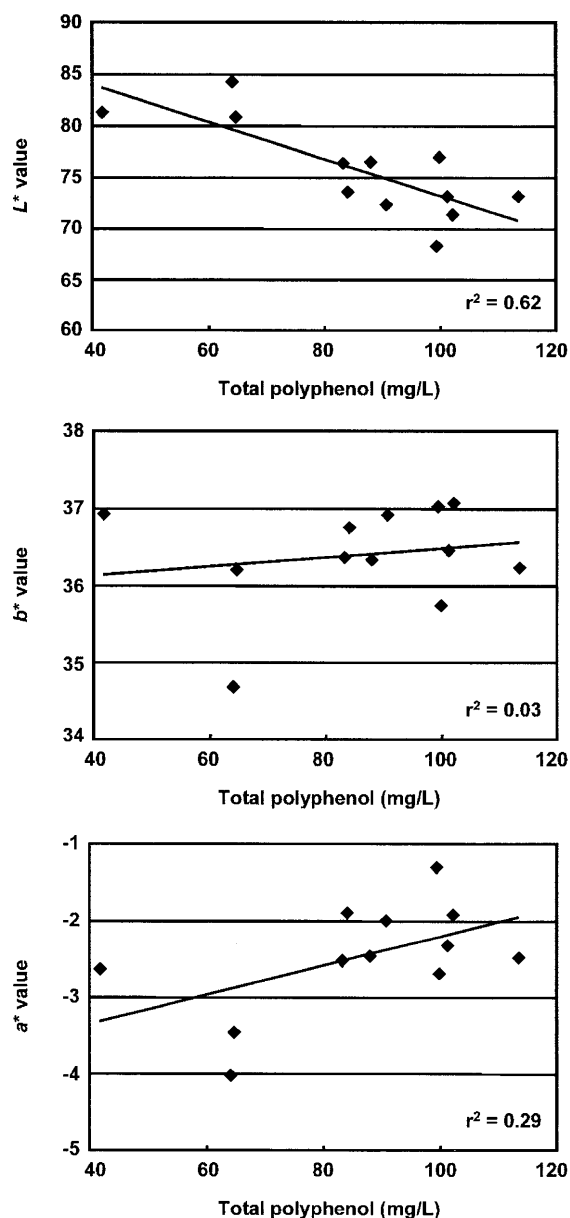


Fig. 6 Correlation between total polyphenol content in Geranium Herb and color indices of Geranium Herb water extract treated with the iron (III) chloride TS. Correlative coefficients are shown in each graph.

characteristic like taste or scent for quantity evaluation. The results obtained by this study point out that it can be applied to evaluation of the total polyphenol quantity of the Geranium Herb more simply and quantitatively by putting the identification test of Geranium Herb in the JP together with the color measurement.

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Japanese abstract

我々は今回、下痢などに対処する整腸剤として、古くから用いられている民間薬ゲンノショウコについて、色彩計を用いた品質評価を行った。ゲンノショウコが示す止瀉作用はポリフェノールの一種であるタンニン類によるものとみなされている。本研究では、ゲンノショウコサンプル中の総ポリフェノール含量と、ゲンノショウコの粉末及び塩化鉄(III)試液を添加したゲンノショウコ熱水抽出液色の関係について検討を行った。その結果、塩化鉄(III)試液を添加したゲンノショウコ熱水抽出液色の明度(L^*)が高い試料ほど、総ポリフェノール含量が低い傾向が見られた。また、総ポリフェノール含量が高い葉の部分では明度は低く、総ポリフェノール含量が低い茎の部分では明度は高かった。従って、ゲンノショウコ中に含まれるタンニン類の作用を期待する場合、茎部分の割合が低いものが良品と考えられ、その評価に、塩化鉄(III)試液を添加したゲンノショウコ熱水抽出液色の明度を利用することが可能であることが示唆された。

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