

## Studies on Bathing Agent I. Anti-inflammatory Effect of Bathing Agent Used for Skin Disease<sup>1)</sup>

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Twenty bathing agents, generally considered to be effective for skin diseases and related symptoms were examined for their effects on skin diseases. Of them, Aoki (*Aucuba japonica*: leaves), Sekisyou (*Acorus gramineus*), Yoobaihi (Myricae cortex), and Sidareyanagi (*Salix babylonica*: leaves and branches) were shown to have an anti-inflammatory effect when used as bathing agent, though the effect varied depending on the extraction temperature: Boiling water extracts of Aoki and Sekisyou had little effect. Sugina (*Equisetum arvense*), Syoubu (*Acorus calamus*: ground parts), Sekisyou (ground parts) and Sidareyanagi were shown to improve blood circulation. Aoki, one of the most effective agents, was further investigated to identify the active component.

**Keywords**—bathing agent; skin disease; *Aucuba japonica*; *Acorus gramineus*; Myricae cortex; *Salix babylonica*; *Acorus calamus*; anti-inflammatory effect; platelet aggregation

Medical bath, or a bath with medicinal plants has been popular for a long time and is said to have good effects on blood circulation and body warming, activating metabolism (effect on women's diseases caused by blood stagnancy, or so-called OKETSU syndrome) and increasing appetites, and heating effects on various skin diseases including miliaria, rough skin and eczema, neuralgia, rheumatism and bruise, and internal diseases or diseases in stomach and intestine. However, the medicinal plants or bathing agents used are traditional folk medicines and little scientific studies have been conducted on their effects and effective components. Generally speaking, any plants with pleasant scent seems to be used as a bathing agent. However, among the plants we examined in the present study were some which were proved to have potent medicinal effects on skin diseases.

### Materials and Methods

**Materials**—The following 20 materials which have been said to be effective in treating swollen and rough skin were used. Myricae cortex (yoobaihi) was obtained commercially, but others were fresh materials collected by us.

Syoubu (*Acorus calamus* L.), Sekisyou (*A. gramineus* SOLAND), Matatabi (*Actinidia polygama* (SIEB. et ZUCC.) MAXIM.), Yomogi (*Artemisia princeps* PAMP.), Aoki (*Aucuba japonica* THUNB.), Kisasage (*Catalpa ovata* G. DON), Sugina (*Equisetum arvense* L.), Uikyoku (*Foeniculum vulgare* MILL.), Gennosyouko (*Geranium thunbergii* SIEB. et ZUCC.), Kakidoushi (*Glechoma hederacea* L. var. *grandis* KUDO), Dokudami (*Houttuynia cordata* THUNB.), Mukuge (*Hibiscus syria* L.), Tuwabuki (*Ligularia tussiliaginea* MAKINO), Yoobaihi (*Myrica rubra* SIEB. et ZUCC.), Oobako (*Plantago asiatica* L.), Hanamomo (*Prunus persica* BATSCH. forma), Sidareyanagi (*Salix babylonica* L.), Yukino-sita (*Saxifraga stolonifera* MEERB.), Irakusa (*Urtica thunbergiana* SIEB. et ZUCC.), Sansyo (*Zanthoxylum piperitum* (L.) DC.).

**Sample preparation**—Normally, a bathing agent is added to water of around 40°C. It is more desirable to immerse herbs in advance in boiling water in a bucket to facilitate more efficient extraction of components, and add the extract to bath. However, some of the active herb components may be volatile and be lost during such extraction with heating. Accordingly, in the present experiment, test samples for screening were prepared by

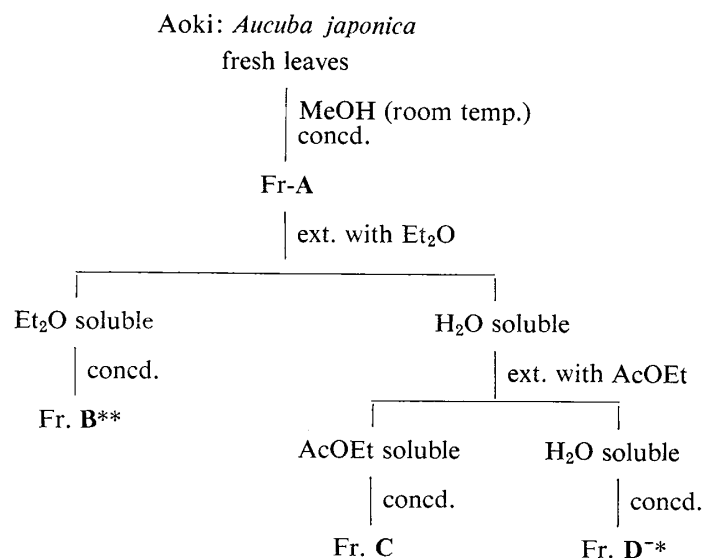


Chart 1. Significantly different inhibitory effect on carrageenin edema from control,  
\*  $p < 0.05$ , \*\*  $p < 0.01$  (at 2 h after carrageenin injection)

extracting the materials either with warm water of around 40°C or with boiling water above 90°C and concentrating the extract *in vacuo* or freeze-drying. Only Aoki was extracted with MeOH along with warm and hot water, as some of its components changed and turned black when water was used as an extracting solvent.

**Inhibition of carrageenin-induced foot edema**—4–5 male Wistar rats of 5-weeks old were used in one group. Carrageenin ( $\lambda$ -carrageenin-PICNIN-A, Zushikagaku Lab., Inc.) was injected into the sole of one leg and an alcoholic solution or suspension of a test sample was applied to the foot surface 4 times, *i.e.* 1 hour before, at the time of, and 1 and 2 hours after the carrageenin injection. The foot volumes were measured at 1, 2, 3 and 4 hours after the carrageenin injection. To control animals, alcohol was applied. The rate of edema inhibition was calculated according to the previous report.<sup>2)</sup>

**Inhibition of histamine-induced contraction**—This screening test was conducted with excised guinea-pig ileum according to the previous report.<sup>3)</sup>

**Inhibition test of platelet aggregation**—This screening test was conducted according to the previously reported method<sup>4)</sup> by using human plasma.

**Fractionation of MeOH extract from Aoki**—The leaves of freshly collected *Aucuba japonica* were extracted with MeOH at room temperature and the extract was concentrated *in vacuo* to give an extract (Fr. A). Fr. A was partitioned between H<sub>2</sub>O and Et<sub>2</sub>O to give an Et<sub>2</sub>O soluble fraction (Fr. B) and a H<sub>2</sub>O soluble fraction. The H<sub>2</sub>O soluble fraction was further extracted with AcOEt to give an AcOEt soluble fraction (Fr. C) and a H<sub>2</sub>O soluble fraction (Fr. D) (Chart 1).

## Results and Discussion

### Inhibitory effect on carrageenin-induced foot edema

Medical bath may be considered to be medicines for external application. Therefore, the extracts were tested for their inhibitory effect on carrageenin-induced rat paw edema (CPE) by local application. As shown in TABLE I, a strong antiphlogistic effect was shown by the warm water extract of Aoki (leaves), Sekisyou (ground part and root), Yoobaihi (Myrica cortex) and Sidareyanagi (mix of branches and leaves). Fresh leaves of Aoki readily turn black on standing after collection. So, dried one, turned black, were also examined and found to be usable though its effect decreased a little. Boiling water often seemed to inactivate the anti-inflammatory effect of plants (for example, Aoki and Sekisyou). So, bathing agents should be more effective if they are added directly to bath tub when an antiphlogistic effect is desired.

### Inhibitory effect on histamine-induced contraction

One way of evaluating anti-inflammatory effect of an agent is to examine the agent for its inhibitory effect on histamine-induced contraction of isolated guinea pig ileum (HCI). Because, histamine is known to be involved in the CPE test as a chemical mediator and, the inhibitory effect on HCI to be parallel with the anti-inflammatory effect on CPE in rats.<sup>3)</sup> The HCI test needed a smaller amount of samples than CPE test. In this experiment, Sekisyou (root) and Oobako showed a strong inhibitory

TABLE I. Inhibitory Effect of Extracts<sup>a)</sup> on Carrageenin-induced Paw Edema in Rats<sup>b)</sup>

Materials (part)	Extraction <sup>c)</sup>	Inhibition (%) of swelling			
		1 h	2 h	3 h	4 h <sup>d)</sup>
Sekisyou (aerial)	w	45.7 <sup>e)</sup>	21.7	31.8	26.4
(do.)	h	-4.3	8.4	5.5	-2.5
(root)	w	1.3	22.7	25.9 <sup>f)</sup>	30.1 <sup>f)</sup>
Aoki (leaves)	w	-0.4	25.0 <sup>e)</sup>	22.6 <sup>e)</sup>	20.1 <sup>e)</sup>
(do.)	h	-34.5	-1.4	1.3	-11.7
(do.)	r.t.	52.4 <sup>f)</sup>	50.8 <sup>f)</sup>	57.4 <sup>f)</sup>	59.0 <sup>f)</sup>
(dry leaves)	w	6.6	22.4 <sup>e)</sup>	23.9 <sup>e)</sup>	3.7
Yoobaihi	w	18.5	24.2 <sup>e)</sup>	33.2 <sup>e)</sup>	29.5 <sup>e)</sup>
Sidareyanagi (branches and leaves)	w	4.7	26.2	32.7 <sup>e)</sup>	35.4 <sup>e)</sup>

<sup>a)</sup> Dose: 5 mg/site  $\times$  4. <sup>b)</sup>  $n=4$  or 5.<sup>c)</sup> w: with H<sub>2</sub>O at 40–42°C, h: with H<sub>2</sub>O at >90°C, r.t.: with MeOH at room temperature<sup>d)</sup> Hours after carrageenin injection.<sup>e)</sup> and <sup>f)</sup>: Significantly different from control, <sup>e)</sup>  $p < 0.05$ , <sup>f)</sup>  $p < 0.01$ .TABLE II. Inhibitory Effect of Extracts on Collagen<sup>a)</sup>-induced Platelet Aggregation

Materials	(part)	Extraction <sup>b)</sup>	Inhibition <sup>c)</sup> %
Sugina	(aerial)	w	91.9
	(do.)	h	96.8
Syoubu	(aerial)	w	95.0
	(do.)	h	96.0
	(root)	w	47.4
	(do.)	h	26.3
Sekisyou	(aerial)	w	94.7
	(do.)	h	92.9
	(root)	w	-62.5
	(do.)	h	-50.0
Sidareyanagi	(leaves)	w	23.4
	(do.)	h	87.5
Gennosyouko	(aerial)	h	55.6
Oobako	(aerial)	h	45.0 <sup>d)</sup>

<sup>a)</sup> at  $10^{-7}$  g/ml.<sup>b)</sup> With H<sub>2</sub>O at 40–42°C (w), at >90°C (h).<sup>c)</sup> at  $10^{-4}$  g/ml.<sup>d)</sup> at  $2 \times 10^{-4}$  g/ml.

effect, but none of the other materials did. (data not shown).

#### Inhibitory effect on platelet aggregation

Inhibitory effect on platelet aggregation may be used for the evaluation of antiphlogistic effects as well as for evaluation of blood circulation improving effect. A strong effect was shown by hot water and warm water extracts of Sugina, Syoubu (ground part) and Sekisyou (ground part) and hot water extract of Sidareyanagi and weak effect was observed in Gennosyouko and Oobako extracts (TABLE II). These plants are thus considered to have effects on improving blood circulation and heating effects on skin diseases. In this case, boiling-water extracts were also effective.

#### Active fraction of Aoki

Of bathing agents examined in this work, Aoki and Sekisyou (root) showed a comparatively strong inhibitory effect on carrageenin-induced paw edema. So, we tried to separate the active substances of

TABLE III. Inhibitory Effects of Fractions from MeOH Extract (F. A) of Aoki on Carrageenin-induced Paw Edema in Rats<sup>a)</sup>

Fractions	Dose (mg/site)	Swelling percent			
		1 h	2 h	3 h	4 h <sup>b)</sup>
EtOH <sup>c)</sup>		21.8 ± 2.33	52.1 ± 2.16	58.0 ± 4.82	59.2 ± 5.89
A	3 mg × 4	14.4 ± 2.95	40.1 ± 3.45*	44.3 ± 4.14	49.0 ± 5.52
B	3 mg × 4	10.5 ± 1.67*	34.7 ± 2.59**	35.7 ± 1.49*	37.6 ± 1.29*
C	3 mg × 4	36.9 ± 3.60*	50.7 ± 6.57	62.3 ± 7.29	60.0 ± 5.59
D	3 mg × 4	33.5 ± 2.15*	63.8 ± 1.42*	64.0 ± 5.16	65.3 ± 8.43
Indomethacin	0.5 mg × 4	19.3 ± 3.82	40.0 ± 4.79	40.9 ± 2.59*	38.9 ± 1.40**

<sup>a)</sup>  $n=4$  or 5. <sup>b)</sup> Time after injection of carrageenin.

<sup>c)</sup> control (0.1 ml × 4/site). \*  $p < 0.05$ , \*\*  $p < 0.01$ .

Aoki. When a warm water or MeOH extract of Aoki (fresh leaves) (Fr. A) was fractionated according to Chart 1, the activity of Fr. A was transferred to lipid soluble fraction (Fr. B) indicating that components other than iridoid glycoside, a main component of the plant, were participating in the anti-phlogistic activity. The water soluble fraction (Fr. D) had an aggravating effect on swollen skin (TABLE III).

We believe that bathing agents, even if they have been popular among people for a long time, should not be used casually without any scientific support. It is noteworthy that the popular bathing agent Syoubu exhibited little anti-inflammatory effect on skin diseases, whereas Sekisyou had a much stronger anti-inflammatory effect. Probably, more easily available Syoubu has replaced Sekisyou, used originally as a medical bathing agent.

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#### References and Notes

- 1) A part of this work was presented at the 36th Annual Meeting of the Japanese Society of Pharmacognosy, Kobe, Sep. 1991.
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