Evaluation of Propolis (II): Effects of Brazilian and Chinese Propolis on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 and Concana valin A

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To establish a biological method for evaluating propolis and to reveal their anti-allergic action, the effects of the ethanol and water extracts (EA-ET and WA-WT, respectively) from Brazilian, Chinese and Japanese propolis on the histamine release induced by compound 48/80 and concana valin A (Con A) were investigated. The relation between the inhibitory activities of these extracts on the histamine release and their E1%cm values were also examined. As a result, the following was found: 1) 0.003—0.01% ethanol and 0.03—0.1% water extracts inhibited the histamine release induced by compound 48/80 and Con A, and the inhibitory potencies of the former extracts were more than 10 times stronger than those of the latter extracts, making it clear that both the ethanol and water extracts possess an anti-allergic action; 2) most of the ethanol and water extracts responded to the histamine release induced by both the histamine releasers in a concentration-dependent manner; 3) the inhibitory activities of 0.003% EM from Hebei Province, EP from Sichuan Province, EQ from Zhejiang Province and ER from Anhui Province in China were weaker than those of 0.01% corresponding extracts, whereas 0.001% ED-EH from Brazilian propolis, EM, EN from Henan Province in China and EP-EH promoted the Con A-induced histamine release of more than 10%, suggesting that such extracts must be carefully given to humans; 4) the inhibitory potencies of only 0.03—0.1% water extracts from Chinese propolis on the Con A-induced histamine release related excellently with their E1%cm values; 5) from the results of the relation between the inhibitory potencies of the propolis extracts and their E1%cm values, it was suggested that an unknown compound, being a poorly water-soluble compound which is a non-flavonoid, with an anti-allergic action is contained in propolis; 6) to precisely evaluate the anti-allergic action of the propolis, the biological method, which measures the inhibitory activities of the propolis extracts on histamine release, was markedly superior to the physicochemical method.

Key words propolis evaluation; ethanol extract; water extract; inhibitory activity; histamine release; coefficient correlation

Honeybees collect exudates from various plants and mix them with bees wax to form a sealing material of certain consistency, called "propolis." Propolis is hard and wax-like when cool, but softens and turns resinous and sticky when warm it is also called bee-glue. Propolis has been used in folk medicines from ancient times in many countries of the world, and has been extensively studied in Eastern European countries.1) Propolis has, to date, been taken in internal and external dosage forms for the treatment of various diseases.1—4) Peterson has demonstrated the strong sensitizing potency of propolis in guinea pigs by the external use of propolis.5) After that, many papers on contact dermatitis have been reported by various investigators.6) In fact, the contact dermatitis has been induced in people who use propolis as an external application and in bee-keepers who have constant contact with propolis when collecting the honey and cleaning the hives.7) In spite of warnings from several investigators, a number of cases of propolis instigated contact dermatitis have been observed related to the external use of propolis. However, there is no information on an anti-allergic activity of propolis.

Generally, it is stated that propolis contains about 30% wax, 55% resins and balsams, 10% therae oils and approximately 5% pollen.8) Furthermore, many constituents have been identified, most being flavonoid aglycones, phenolic acids and their derivatives.9) Among these constituents, it is thought that the compound which induces contact dermatitis is caffeic acid phenylester.10) The proportion of these constituents is dependent on the place and time propolis is collected. Accordingly, it is very important to check the quality of propolis by various biological evaluations to prevent a crisis of adverse effects such as contact dermatitis. However, information concerning the biological evaluation of propolis is lacking. From this view, in a previous paper11) we investigated the anti-hyaluronidase activities of various propolis extracts, and evaluated them by their enzyme inhibitory activities and physicochemical data, which included their absorption spectra and specific absorbance (E1%cm) values. Furthermore, the relation between the anti-hyaluronidase activities of these extracts and their E1%cm values was studied.

In this paper, to reveal whether various propolis from different countries and plant sources induce or inhibit an allergic reaction, we studied the effects of 99.5% ethanol and water extracts from Brazilian, Chinese and Japanese propolis on the histamine release from rat peritoneal mast cells induced by compound 48/80 and concanavalin A (Con A). In addition, the relation between the inhibitory activities of these extracts on the histamine release and their E1%cm values was also examined.

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MATERIALS AND METHODS

Materials Ninty-nine point five (99.5)% ethanol and the water extracts described below were the same as those used in the previous paper.\(^1\) Propolis A-L from Brazil (A-B: Araucaria angustifolia (BERT) O. KTZE; C-I: Eucalyptus globulus L.; J-I: Rosmarinus officinalis L.), propolis M-S from China (M: Hebei Province; N: Henan Province, O: Jiangsu Province; P: Sichuan Province; Q: Zhejiang Province; R: Anhui Province; S: Hubei Province), and propolis T from Japan (Akita Prefecture) were used. 99.9% Ethanol and water extracts from propolis A-T were named EA-ET and WA-WT, respectively. Compound 48/80, Con A and heparin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Trichloroacetic acid was purchased from Wako Pure Chemical Co., Osaka. N-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) was purchased from Dojin Do Laboratories, Kumamoto, Japan. All other reagents were of analytical grade.

Preparation of Propolis Solution Each solution of propolis extracts was prepared according to the methods described in the previous paper.\(^1\)

Measurement of Fluorescence Intensity Fluorescence intensity was measured with a Hitachi fluorescence photometer F-3000.

Animals Male Wistar rats (200—220 g), purchased from Charles River, Japan, were used. They were housed in raised mesh-bottom cages under conditions of 22.2 °C temperature, 55.5% humidity and 12 h light (from 7 a.m. to 7 p.m.), and were given a commercial pellet diet (MF, Oriental Yeast Co., Ltd., Tokyo) and allowed tap water ad libitum. Before the experiments, the rats were starved for 24 h but allowed free access to water.

Isolation of Rat Peritoneal Mast Cells Male Wistar rats were exsanguinated and injected intraperitoneally with 20 ml of physiological solution (PSS (-) glucose) consisting of 145 mM NaCl, 2.7 mM KCl, 1.0 mM CaCl\(_2\), 5 mM HEPES, and 5 mM phosphate buffer, pH 7.4. The abdominal region was gently massaged for 2 min and the peritoneal exudate cells were collected in a siliconized glass vessel. The cell suspension was centrifuged (265×g for 3 min at 4 °C) and washed several times with the physiological solution.

Assay of Histamine Release from Rat Peritoneal Exudate Cells Induced by Compound 48/80 and Con A A 0.05 ml portion of the propolis extract dissolved in dimethyl sulfoxide (DMSO) was mixed with 1.7 ml of physiological solution (PSS (+) glucose) consisting of 145 mM NaCl, 2.7 mM KCl, 1.0 mM CaCl\(_2\), 5 mM HEPES, 5.6 mM glucose, and 5 mM phosphate buffer, pH 7.4. The mixed solution was preincubated at 37 °C for 5 min, then 0.05 ml of peritoneal exudate cell suspension was added, and the mixture was incubated at 37 °C for 15 min. The test substance was replaced by physiological solution as a control. The preincubated peritoneal cell suspension was mixed with 0.2 ml of compound 48/80 (1.0×10\(^{-5}\) g/ml) or Con A (4×10\(^{-4}\) g/ml) solutions, and incubated at 37 °C for 10 min. The physiological solution was added in place of compound 48/80 or Con A solutions as a blank. The mixture was cooled to 4 °C and centrifuged at 3000×g at this temperature for 10 min. Histamine in the supernatant and residue was measured, respectively, according to the method of Shore et al.\(^1\) This assay was done 3—5 times per sample. The percent inhibition was calculated as follows.

\[
\text{histamine release} = \frac{P_r}{P_r + P_f} \\
\text{P}_r: \text{histamine in supernatant} \\
\text{P}_f: \text{residue histamine in cells} \\
\text{inhibition} = \left( \frac{S-B}{C-B} \times 100 \right) \\
S: A \text{ obtained from test sample} \\
C: A \text{ obtained from control} \\
B: A \text{ obtained from blank}
\]

RESULTS AND DISCUSSION

Tables 1—4 show the effects of the ethanol and water extracts on the histamine release from rat peritoneal mast cells induced by compound 48/80 and Con A. Compound 48/80, which is a non-specific histamine releaser, releases the histamine from mast cells by disrupting their cell membranes.\(^1\) On the other hand, ConA, a lectin derived from jack bean, is also a histamine releaser which has a different mechanism for histamine release from that of compound 48/80, namely, the mechanism of Con A-induced histamine release from mast cells is similar to that induced by an antigen-antibody reaction.\(^4\) So, we investigated how the ethanol and water extracts from various propolis inhibit the histamine release induced by both the stimuli to reveal the anti-allergic action of propolis and to establish a biological method of evaluating propolis.

Ethanol Extracts Effects on Compound 48/80-Induced Histamine Release: First, we investigated whether various ethanol extracts inhibited the histamine release induced by compound 48/80. Table 1 shows the effects of the 0.001—0.01% ethanol extracts from Brazilian, Chinese and Japanese propolis on the histamine release. One-thousandth to 0.01% ethanol extracts from Brazilian and Japanese propolis inhibited the compound 48/80-induced histamine release in a concentration-dependent manner, but those extracts from Chinese propolis did not. All the extracts used, in 0.003—0.01% concentrations, inhibited the histamine release. The inhibition of many 0.001% ethanol extracts was below 15%. In the 0.003% concentration, the ethanol extracts from Chinese propolis, except for EM, showed over 80% inhibition, which was larger than those of 0.003 % extracts from Brazilian and Japanese propolis, and those inhibitory potencies were almost the same as those of 0.01% EN, EO and ES 100% EM, EP and EQ inhibited it less 75%, which was smaller than those of 0.003% corresponding extracts.

Effects on Con A-Induced Histamine Release: Next, we examined the effects of the ethanol extracts on the histamine release induced by Con A. Table 2 shows the results. In 0.003—0.01% concentrations, these extracts inhibited Con A-induced histamine release over 50%. EA, EC, EE-EJ, EJ and EL from Brazilian propolis responded concentration-dependently to the histamine release. By changing the histamine releaser from compound 48/80 to Con A, the behavior of 0.001% ED-EH (from Eucalyptus Globulus L. in Brazil), EM, EN, EP and ER (from Chinese propolis) on the histamine release was changed to promote it over 10%, especially
EN from Henan Province in China, which promoted it 44%. Other ethanol extracts had no significant effect on the release. Most of the 0.003% ethanol extracts from Brazilian propolis inhibited the Con A-induced histamine release more than those induced by compound 48/80, and all 0.01% extracts showed inhibitory activities over 65%, as same as using compound 48/80 as a histamine releaser. As with compound 48/80, the inhibitory effects of 0.003% EM and EP-ER from Chinese propolis on the Con A-induced histamine release were more potent than those of 0.01% corresponding extracts. This phenomenon was observed in ET from Japanese propolis, indicating a different inhibitory profile in the case of compound 48/80. Three thousandth of a percent EB, ED, EH, EI, KK (from Brazilian propolis) and EN, EO, ES (from Chinese propolis) inhibited the release as potently as those in the 0.01% concentration.

In the previous paper, we reported that among the ethanol extracts from Brazilian propolis, EA and EB (from *Artocarpus angustifolia* (Berk.) O. KTZE) inhibited the hyaluronidase activities less than EC-EI (Eucalyptus globulus L.) and EJ-EL (Rosmarinus officinalis L.), indicating that the enzyme inhibitory activities of these extracts were dependent on the plant sources of propolis collected. This result was thought to be based on the lower *E*<sub>100</sub> values of EA and EB than those of other ethanol extracts. The effects of 0.001—0.01% ethanol extracts from Chinese propolis (EM-ES) on the histamine release induced by both the releasers varied clearly by the location of propolis collection. The inhibitory activities of 0.003% EM and EP-ER on the histamine release induced by both the releasers were more potent than those of 0.01% corresponding extracts. Namely, these extracts showed bell-shaped inhibitory profiles on the histamine release. This suggested that EM and EP-ER contained two kinds of compounds with inhibiting and accelerating actions on histamine release. The inhibitory abilities of 0.001% ethanol extracts from Brazilian propolis on the Con A-induced histamine release (Tables 1 and 2) were dependent on the plant sources of propolis collection.

These results showed that 0.003—0.01% ethanol extracts from Brazilian, Chinese and Japanese propolis clearly possessed an anti-allergic action. However, with respect to the ethanol extract from Chinese propolis, their inhibitory effects on histamine release must be examined in various concentrations, at least in the range of 0.001—0.01% concentrations, because their inhibitory potencies at a 0.01% concentration were less than those in 0.003% concentration. So, such extracts should not be given in excess to humans because they might reduce an anti-allergic action or might actually induce an allergic reaction. Furthermore, many ethanol extracts of a lower concentration (0.001%) must be carefully given to human and must not be used as an external drug because they promoted the histamine release induced by Con A, resulting in the possibility of a crisis of allergic reaction when such extracts were given orally.

**Water Extracts**  Effects on Compound 48/80-Induced Histamine Release: Table 3 shows the values of percent inhibition of the water extracts on the compound 48/80-induced histamine release. The water extracts gave concentration-response curves on the histamine release induced by compound 48/80 at 0.01—0.1% concentrations. However, at a lower concentration (0.01%), most of the water extracts inhibited the histamine release less than 20%. The inhibitory activities of 0.03% water extracts were about 10—80%, especially, WD, WO and WP inhibited it more than 60%. Focusing on the water extracts from Chinese propolis, WO and WP exhibited 2—4 times stronger inhibitory effects than other water

<table>
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<tr>
<th>Extract</th>
<th>Specific absorbance (E&lt;sub&gt;100&lt;/sub&gt; value&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Inhibition (%)</th>
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<tr>
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<td>Concentration (%)</td>
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<tr>
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<td>EE 375 (294.4)</td>
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<td>EF 278 (295.6)</td>
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<td></td>
<td>EG 323 (297.2)</td>
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<tr>
<td>J</td>
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<table>
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<tr>
<th>Extract</th>
<th>Specific absorbance (E&lt;sub&gt;100&lt;/sub&gt; value&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Inhibition (%)</th>
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<tr>
<td></td>
<td>Concentration (%)</td>
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<tr>
<td>B</td>
<td>EA 170 (238.2)</td>
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<td>EB 156 (272.0)</td>
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<td>J</td>
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<td>11</td>
</tr>
</tbody>
</table>

<sup>a</sup> The values were measured at a wavelength of each absorption maximum (nm) presented in parentheses. B, Brazil; C, China; J, Japan.
Table 3. Inhibitory Effects of Water Extracts on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

<table>
<thead>
<tr>
<th>Extract</th>
<th>Specific absorbance ($E_{1cm}^{1cm}$ value)$^a$</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (%)</td>
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</tr>
<tr>
<td>B WA</td>
<td>74 (279.6)</td>
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</tr>
<tr>
<td>WB</td>
<td>108 (280.0)</td>
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<tr>
<td>WC</td>
<td>101 (285.6)</td>
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</tr>
<tr>
<td>WD</td>
<td>131 (287.2)</td>
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<tr>
<td>WE</td>
<td>178 (289.2)</td>
<td>12</td>
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<tr>
<td>WF</td>
<td>102 (287.6)</td>
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<td>WH</td>
<td>116 (286.8)</td>
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<td>WI</td>
<td>112 (286.4)</td>
<td>1</td>
</tr>
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<td>WJ</td>
<td>131 (287.2)</td>
<td>7</td>
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<tr>
<td>WK</td>
<td>135 (287.2)</td>
<td>19</td>
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<tr>
<td>WL</td>
<td>151 (288.0)</td>
<td>8</td>
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</table>

| C WM    | 208 (287.2)                                   | 14    | 39    | 92    |
| WN      | 204 (286.0)                                   | 1     | 42    | 93    |
| WO      | 188 (286.8)                                   | 13    | 70    | 100   |
| WP      | 203 (289.6)                                   | 7     | 82    | 97    |
| WQ      | 195 (286.8)                                   | -1    | 19    | 97    |
| WR      | 172 (291.2)                                   | 21    | 41    | 82    |
| WS      | 181 (286.4)                                   | 7     | 29    | 72    |
| J WT    | 225 (286.8)                                   | 12    | 38    | 77    |

$^a$ The values were measured at a wavelength of each absorption maximum (nm) presented in parentheses. B, Brazil; C, China; J, Japan.

Extracts. These results indicated that the inhibitory abilities of 0.03% water extracts from Chinese propolis were dependent on different locations of propolis collection. In contrast, 0.03% WA-WL from Brazilian propolis, which were obtained from three kinds of plant sources, could not be characterized by the plant sources of propolis collection. At a 0.1% concentration, all water extracts inhibited the histamine release over 70%.

Effects on ConA-Induced Histamine Release: Table 4 shows the values of percent inhibition of the water extracts on ConA-induced histamine release. In this case, the water extracts inhibited, concentration-dependently, the histamine release except for WC, WD and WR. At a 0.01% concentration, WB, WE, WP, WQ, and WT inhibited Con A-induced histamine release about 20—60%, and it was noticeable that the inhibitory potency of 0.01% WN was 50%, whereas 0.01% WG and WI promoted histamine release more than 10%. The inhibitory activities of 0.03% WE, WG, WN, WP and WT on the release were more than about 50%. One-tenth percent WE, WG, WK and WM-WP inhibited the release over 85%, whereas other water extracts inhibited it less than 60%. Among 0.1% water extracts from Chinese propolis, the inhibitory activities of WR and WS were markedly weak compared with those of WN-WQ, indicating that the inhibitory effects of propolis extract vary by the location of propolis collection. At the higher concentration (0.1%), the water extracts from Chinese propolis, except for WR and WS, showed stronger inhibitory effects than those from Brazilian propolis, except for WR and WS, in spite of approximately the same $E_{1cm}^{1cm}$ values. In this way, the inhibitory activities of 0.03 and 0.1% water extracts from Chinese propolis were dependent on the locations of propolis collection. From these results, it was found that many propolis extracts inhibited the histamine release induced by both compound 48/80 and Con A. In this connection, Khayal et al. demonstrated that 13% water extracts from propolis did not affect the histamine release from perfused guinea-pig lung.15

The inhibitory activities of WB, WE, WG and WK (from Brazilian propolis, WM-WQ (from Chinese propolis) and WT (from Japanese propolis) were almost similar to each other on both the releasers-induced histamine release, but other water extracts showed stronger inhibitory activities on the release induced by Con A than those induced by compound 48/80. From comparing the inhibitory activities of the ethanol extracts with those of the water extracts on the release induced by compound 48/80 and Con A, it was found that many water extracts exhibited more than 10 times weaker inhibitory effects than those of the corresponding ethanol extracts.

These findings indicated that many 0.03—0.1% water extracts had a clear anti-allergic action although they were weaker than those of the ethanol extracts. The water extracts from Chinese propolis varied in effect on histamine release according to plant collection location, but those from Brazilian propolis did not show inhibitory profiles characterized by their locations. In addition, as most of the water extracts gave concentration-dependent response curves on the release induced by both the releasers, not much attention to their dosage may be needed, which differs from the ethanol extracts from Chinese propolis.

Relationship between Inhibitory Activities of Propolis Extracts on Histamine Release and Their $E_{1cm}^{1cm}$ Values: In the previous paper11 we examined the relation between the hyaluronidase inhibitory activities of various propolis extracts and their $E_{1cm}^{1cm}$ values. As a result, it was found that the enzyme inhibitory activities of ethanol extracts and their...
Fig. 1. Relation between Inhibitory Activities of Ethanol Extracts on Compound 48/80-Induced Histamine Release and Their $E_{1\text{cm}}^{1\%}$ Values
Concentrations of ethanol extracts: A, 0.003%; B, 0.01%.

Fig. 2. Relation between Inhibitory Activities of Ethanol Extracts on Con A-Induced Histamine Release and Their $E_{1\text{cm}}^{1\%}$ Values
Concentrations of ethanol extracts: A, 0.003%; B, 0.01%.

$E_{1\text{cm}}^{1\%}$ values bore a linear relation to each other, but those activities of the water extracts decreased with an increase in the $E_{1\text{cm}}^{1\%}$ values. In this way, both the extracts showed different profiles in relation to each other. So, to examine the relation between the inhibitory activities of various propolis extracts on the histamine release and their $E_{1\text{cm}}^{1\%}$ values, the values of % inhibition in various concentrations on compound 48/80- and Con A-induced histamine release were plotted against $E_{1\text{cm}}^{1\%}$ values shown in Table 1 for the ethanol extracts and in Table 3 for the water extracts. Figures 1—4 depict the re-
Table 5. Correlation Coefficients between Inhibitory Abilities of 99.5% Ethanol Extracts on Compound 48/80- and Concanavalin A-Induced Histamine Release and Their $E_{1cm}^{1\%}$ Values

<table>
<thead>
<tr>
<th>Ethanol extract</th>
<th>Correlation concanavalin</th>
<th>Correlation concanavalin</th>
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<tr>
<td></td>
<td>Compound 48/80</td>
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<tr>
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<td>Concentration of extract (%)</td>
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<td>EA-ET</td>
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<td>0.548</td>
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<td>EA-EL (from Brazilian propolis)</td>
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<td>-0.313</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.581</td>
</tr>
</tbody>
</table>

Table 6. Correlation Coefficients between Inhibitory Abilities of 99.5% Water Extracts on Compound 48/80- and Concanavalin A-Induced Histamine Release and Their $E_{1cm}^{1\%}$ Values

<table>
<thead>
<tr>
<th>Water extract</th>
<th>Correlation concanavalin</th>
<th>Correlation concanavalin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound 48/80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration of extract (%)</td>
<td>0.01</td>
</tr>
<tr>
<td>WA-WT</td>
<td>0.202</td>
<td>0.483</td>
</tr>
<tr>
<td>WA-WL (from Brazilian propolis)</td>
<td>0.291</td>
<td>0.370</td>
</tr>
<tr>
<td>WM-WS (from Chinese propolis)</td>
<td>-0.489</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration of extract (%)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.467</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.140</td>
</tr>
</tbody>
</table>

The inhibitory activities of 0.003% ethanol extracts on compound 48/80- and Con A-induced histamine release related moderately to their $E_{1cm}^{1\%}$ values (the $r$ values were 0.548 and 0.417, respectively), but those of 0.01% ethanol extracts varied inversely to their $E_{1cm}^{1\%}$ values. However, the inhibitory activities of 0.03% water extracts on compound 48/80-induced histamine release correlated moderately to their $E_{1cm}^{1\%}$ values ($r=0.483$), but, in the 0.01% concentration, the relation between them was poor ($r=0.136$). In the case of Con A used as a stimulus, differing from the ethanol extracts, the water extracts gave a positive correlation in both 0.03 and 0.01% concentrations ($r=0.479$ and 0.486, respectively).

Next, to elucidate the effect of the location of propolis collection on such a relationship, we examined the relation between the inhibitory activities of Brazilian propolis extracts and Chinese ones, and between their $E_{1cm}^{1\%}$ values, respectively. Only the results on 0.03 and 0.1% water extracts from Chinese propolis are shown in Fig. 5. The inhibitory activities of 0.01% Brazilian ethanol extracts on compound 48/80-induced histamine release were inversely proportional to the $E_{1cm}^{1\%}$ values, with a $-0.539$ $r$ value. In contrast, those of 0.003% Chinese ethanol extracts were increased, with an increase in their $E_{1cm}^{1\%}$ values to a 0.508 of $r$ value. Other extracts did not show a noticeable correlation between their inhibitory activities on both the releasers-induced histamine release and their $E_{1cm}^{1\%}$ values.

One-tenth percent WM-WS from Chinese propolis showed a moderately good relationship ($r=0.604$) between the inhibitory activities of these extracts on the compound 48/80-induced histamine release and their $E_{1cm}^{1\%}$ values, but there was no the clear-cut correlation in other extracts. In the case of Con A used as a stimulus, only 0.03 and 0.1% water extracts from the Chinese propolis showed especially good correlation (Fig. 5 and Table 6). In contrast, the $r$ values of 0.03
and 0.1% of Brazilian water extracts were 0.413 and 0.488, respectively.

Generally, it has been believed that the various pharmacological actions of propolis are attributable to phenolic compounds such as flavonoids and caffeic acids contained in propolis, so propolis has been evaluated by using their $E_{410\text{nm}}^{1\%}$ values which were dependent on the content of those compounds.\textsuperscript{10} The results obtained in this paper indicate that the inhibitory activities of propolis extracts on histamine release are dependent on many factors such as the kind of histamine releaser (compound 48/80 or Con A), the extraction solvent used to obtain propolis extract, the concentrations of propolis extract and the location of propolis collection. Accordingly, the anti-allergic abilities of propolis extracts must be carefully evaluated according to their inhibitory activities on histamine release. In addition, the inhibitory activities of all the propolis extracts on the histamine release induced by both stimuli did not necessarily show a clearly positive correlation to the $E_{410\text{nm}}^{1\%}$ values, and the activities of some ethanol extracts related negatively to the $E_{410\text{nm}}^{1\%}$ values. These data strongly suggest that an unknown compound, which is a poorly water-soluble compound and a non-flavonoid, with inhibitory action on histamine release is contained in propolis.

In conclusion, we found that 0.003—0.01% ethanol extracts and 0.03—0.1% water extracts possessed obvious anti-allergic action. The inhibitory activities of the ethanol extracts on the histamine release induced by both the releasers were more than 10 times stronger than those of the water extracts. The inhibitory activities of the many propolis extracts were dependent on many factors, so the anti-allergic abilities of propolis extracts must be evaluated, not by $E_{410\text{nm}}^{1\%}$ values of propolis extracts, but by their inhibitory activities on histamine release. Further work to isolate the unknown compound with an anti-allergic action is now in progress because the experimental results suggest that such a compound is contained in propolis.

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