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## Cholagogic and Antiulcer Effect of *Saussureae Radix* and Its Active Components

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In order to clarify the stomachic effect of *Saussureae Radix* (Mokko), its effect and its active constituents have been investigated in terms of cholagogic and antiulcer action. It was found that the acetone extract from Mokko and one of the constituents, costunolide, both possess a cholagogic effect and an inhibitory effect on the formation of gastric ulcer (induced by restraint in water) in mice.

**Keywords**—stomachic effect; cholagogic effect; antiulcer; *Saussureae Radix*; acetone extract; costunolide

Mokko (*Saussurea lappa*, a root of genus *Chrysanthemum*) is classified as an aromatic stomachic, and is a component of several crude drugs used as gastrointestinal medicines (Rokushin-gan, Kio-gan, Jitsubo-san). It is also an important fragrance. Mokko is known to contain costunolactone, dehydrocostuslactone, saussurealactone and costunolide, which are essential oils. In addition, it also contains saussurine (an alkaloid) and tannin.<sup>1)</sup>

Although Mokko has been used as an aromatic stomachic and has been classified as such, only its antibacterial effect has been reported<sup>2)</sup> and no study has been done on its stomachic action. We have, therefore, investigated the effects of Mokko and its constituents on the rate of bile secretion and gastric ulcer formation in this work.

### Experimental

#### Materials

Mokko was obtained from a local market in Osaka. It was cut coarsely, macerated in about 10 volumes of acetone or distilled water, and kept for 3 d at room temperature. After filtration, the filtrate was concentrated and dried at 40°C under reduced pressure (dried acetone extract). To obtain a dried water extract, the filtrate was concentrated to about half original volume under reduced pressure (below 50°C), and then freeze-dried.

Since the acetone extract was found to possess activity, the acetone extract was fractionated by column chromatography (silica gel as an absorbent): fraction I (8.5% yield), fraction II (17.8% yield), fraction III (16.8% yield), fraction IV (16.7% yield) and fraction V (40.2% yield). Based on the melting point, and infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometer (MS) measurements, fraction III was found to be costunolide. The thin layer chromatographic (TLC) pattern is shown in Chart 1.

#### Methods

**1. Cholagogic Action**—The method used to assess the cholagogic effect was the same as previously reported.<sup>3)</sup> Male Wistar rats weighing about 300 g were divided into groups of 10. The animals were fasted for 6 h before operation. Each animal was anesthetized lightly with ether, then anesthetized with urethane (700 mg/kg, *i.p.*), and laparotomy was performed to insert a polyethylene cannula (Hibiki) into the common bile duct. Animals were then allowed to rest for 1 h to reach a steady state. After a further 30 min, rats were given a test compound intraduodenally (*i.d.*) in a suspension of 5% acacia. The bile secretion was measured at 0.5, 1, 1.5, 2, 3, 4, and 5 h after administration. The percent changes in bile secretions at each time interval were calculated based on the bile secretion

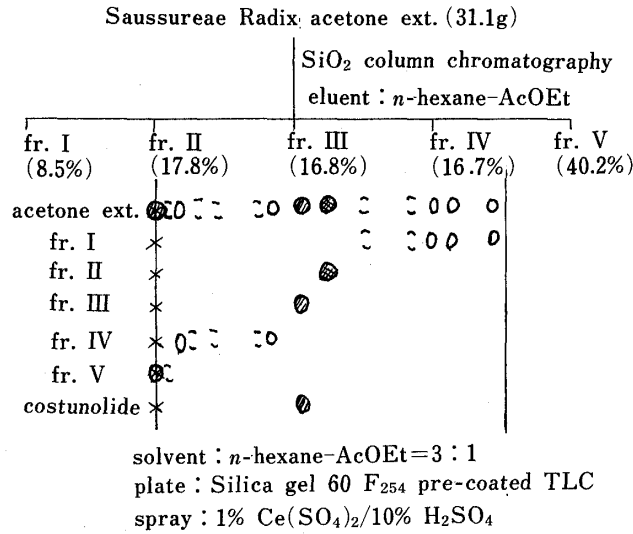


Chart 1. Flow Diagram of Fractionation of Saussureae Radix Acetone Ext.

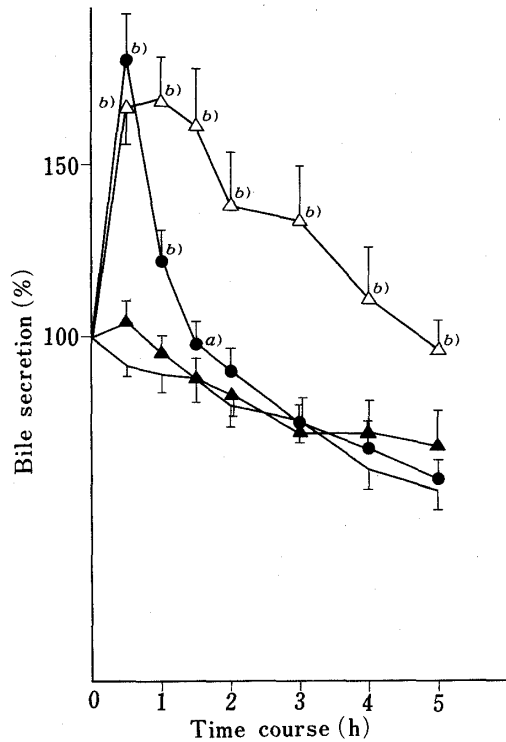


Fig. 1. Effect of Saussureae Radix on Bile Secretion in Rats

—, control; —△—, Saussureae Radix acetone ext. 500 mg/kg; —▲—, Saussureae Radix H<sub>2</sub>O ext. 3000 mg/kg; —●—, sodium dehydrocholate 100 mg/kg.

Each value is the mean with standard error obtained from 10 rats. Significantly different from control at a)  $p < 0.05$ , b)  $p < 0.01$ . Drugs were administered *i.d.* at 0h.

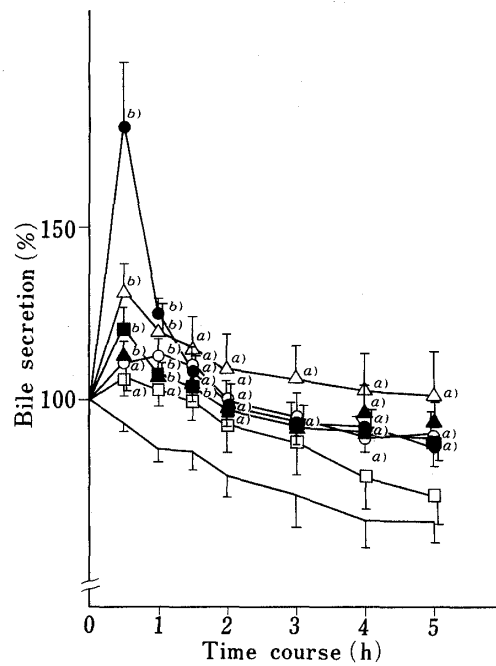


Fig. 2. Effect of Fractions of Saussureae Radix Acetone Ext. and Sodium Dehydrocholate on Bile Secretion in Rats

—, control; —○—, fraction I 100 mg/kg; —▲—, fraction II 100 mg/kg; —△—, fraction III 100 mg/kg; —■—, fraction IV 100 mg/kg; —□—, fraction V 100 mg/kg; —●—, sodium dehydrocholate 100 mg/kg.

Each value is the mean with standard error obtained from 10 rats. Significantly different from control at a)  $p < 0.05$ , b)  $p < 0.01$ . Drugs were administered *i.d.* at 0h. Fraction III, constunolide.

during 30 min before administration of the test compounds as 100%. Sodium dehydrocholate (DC-Na) served as the control drug.

TABLE I. Effect of Saussureae Radix on Gastric Ulcers in 5h Water Immersion-Stressed Mice

Drugs	Dose (mg/kg, <i>p.o.</i> )	Ulcer index (mean $\pm$ S.E.)	Inhibition (%)
Control	—	2.7 $\pm$ 0.2	—
Saussureae Radix acetone ext.	500	0.5 $\pm$ 0.2 <sup>b)</sup>	81.5
Atropine	30	0.6 $\pm$ 0.2 <sup>b)</sup>	77.8
Control	—	2.4 $\pm$ 0.2	—
Fraction I	300	2.0 $\pm$ 0.2	16.7
Fraction II	150	2.3 $\pm$ 0.2	4.2
	300	2.3 $\pm$ 0.2	4.2
Fraction III <sup>c)</sup>	150	2.1 $\pm$ 0.2	12.5
	300	1.6 $\pm$ 0.2 <sup>a)</sup>	33.3
Fraction IV	300	2.0 $\pm$ 0.3	16.7
Fraction V	300	2.4 $\pm$ 0.2	0.0
Atropine-sulfate	30	0.7 $\pm$ 0.2 <sup>b)</sup>	70.8

Drugs were administered *p.o.* before stress. Each value is the mean with standard error obtained from 10 mice. Significantly different from control at a)  $p < 0.05$ , b)  $p < 0.01$ . c) Fraction III (costunolide).

**2. Antiulcer Action**—(a) Gastric Ulcer Induced by Restraint in Water: Groups of 10 male dd-k mice weighing between 18–20 g were used. After 24 h of fasting, each animal was orally (*p.o.*) given a test compound in a suspension of 5% acacia, and immediately put into a pipe (10 cm long, 2 cm i.d.), covered at the top and bottom with metal mesh, and immersed in water (23 °C) to the level of its neck. After 5 h, each animal was removed from the water, and sacrificed. The stomach was excised and fixed in 10% formalin. Each stomach was cut open along the greater curvature, and a macroscopic rating of ulceration was made according to a scoring system based on the number of ulcers and severity in the fundus region.<sup>4)</sup> The results were expressed as percentages in relation to the control.

(b) Gastric Ulcer Induced by Pylorus Ligation<sup>5)</sup>: Male Wistar rats weighing 200–220 g were divided into groups of 10. After 48 h of fasting, the rats were anesthetized with ether, and laparotomy was performed to ligate the pylorus. After 13 h, the animals were sacrificed, and the stomach of each was excised. The volume of the stomach content and the pH were measured by using a graduated cylinder and a pH meter (Horiba digital pH meter, F-7<sub>LC</sub>), respectively. The stomach was then cut open along the greater curvature. The macroscopic rating of ulcer formation was done according to a scoring system based on the number of ulcers and their severity in the forestomach region. The results were expressed as percentages in relation to the control. Statistical analysis was performed by using Student's *t*-test.

## Results

### 1. Cholagogic Action

As shown in Fig. 1, the acetone extract exhibited a significant cholagogic action. No difference was observed in the amount of bile secretion between the acetone extract-treated animals and the DC-Na control within 30 min of administration, but the acetone extract had a significant effect between 30 min and 5 h after administration. The water extract, however, caused no significant increase in the bile secretion as compared to the control between 30 min and 5 h after the administration of the compounds. Since the acetone extract exhibited a significant effect as compared to the water extract, each fraction of the extract was further examined for cholagogic effect. All the fractions examined (fractions I through V) showed some cholagogic activity, and fraction III (costunolide) exhibited the strongest activity at 100 mg/kg, *i.d.* It increased the bile secretion by about 130% at 30 min after administration, and the effect lasted until 5 h after administration.

### 2. Antiulcer Action

(a) Gastric Ulcer Induced by Restraint in Water—The acetone extract (which had a

significant cholagogic effect), 500 mg/kg *p.o.*, had a significant antiulcer action as compared to the control. Among the fractions of the acetone extract, fraction III (costunolide) at 300 mg/kg *p.o.* prevented the formation of ulcers as compared to the control, in a dose-dependent manner. Fractions I, II, IV and V at 300 mg/kg *p.o.* also showed significant antiulcer effects, but their effects were weaker than that of fraction III.

Fraction III (costunolide) was also examined for activity to inhibit pylorus ligation-induced ulcer formation, but showed no beneficial effect of costunolide even at 300 mg/kg *i.d.*

### Discussion

We have previously reported the cholagogic action of clove<sup>3)</sup> and cardamom<sup>6)</sup> classified as aromatic stomachics, and an analysis of their constituents. However, since there are almost no natural medicinal products classified as aromatic stomachics whose effects have been experimentally confirmed, further work is necessary to evaluate the beneficial effects.

The results of the present experiments demonstrate that the acetone extract of Mokko and one of its components, costunolide, possess long lasting cholagogic action in contrast to the short-duration effect of the reference agent, sodium dehydrocholate. In addition, the acetone extract and costunolide significantly inhibited the ulceration induced by restraint in water. Thus, Mokko clearly has some beneficial effects as a stomachic agent.

It has been reported that, among the components of natural medicinal substances, compounds with phenolic<sup>3,7)</sup> and alcoholic<sup>8)</sup> hydroxyl groups, as well as iridoid,<sup>9)</sup> coumarin,<sup>10)</sup> and monoterpene<sup>6)</sup> compounds exhibit cholagogic characteristics. Saponins,<sup>11)</sup> alkaloids,<sup>12)</sup> and flavone<sup>13)</sup> compounds have also been reported to have antiulcer properties. It has been demonstrated in this study that costunolide, a sesquiterpene compounds, possesses both cholagogic and antiulcer properties.

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