Antiinflammatory Effect of *Graptophyllum pictum* (L.) GRIFF.

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The red leaves of *Graptophyllum pictum* (L.) GRIFF. (*G. pictum*) are used in Indonesian folk medicine. The present study was carried out to elucidate the antiinflammatory effect of the ethanol extract obtained from this crude drug. The extract was partitioned between ether and water, and then the water-soluble fraction was extracted with 1-butanol. The 1-butanol-soluble fraction was extracted with chloroform–acetone, hot methanol and water, successively, and the hot methanol-soluble fraction (fr.) was chromatographed (frs. I—III). The antiinflammatory activity of these fractions was investigated on carrageenin-induced edema in rats and acetic acid-induced vascular permeability as well as the writhing symptom in mice. The ethanol extract (*p.o.*) showed an antiinflammatory activity as well as an analgesic activity and these activities shifted to the water-soluble fraction, 1-butanol-soluble fraction, methanol-soluble fraction and fr. II, successively. It was found that fr. II contained flavonoids. These results suggest that these flavonoids are at least partly responsible for the antiinflammatory effect of the ethanol extract of *G. pictum*.

Keywords antiinflammatory effect; carrageenin-induced edema; vascular permeability; *Graptophyllum pictum*; flavonoid

Two types of *Graptophyllum pictum* (L.) GRIFF. (*G. pictum*) are cultivated in Indonesia, one of which has red leaves and the other, green. The red leaves of *G. pictum* are used in Indonesian folk medicine as a poultice on cuts, wounds and all kinds of swellings, as well as for the treatment of ulcer, abscess, hemorrhoids, *etc.*[1-3] Although some pharmacological studies on this crude drug have been reported,[1] little is known about the antiinflammatory activity.

On the basis of the uses in folk medicine, the present study was carried out to elucidate the antiinflammatory effect of a 50% ethanol extract obtained from the red leaves of *G. pictum* and to identify the active principle(s).[4]

Experimental

Materials The red dry leaves of *G. pictum* were refluxed with 50% ethanol for 6 h, three times. The solution was filtered through filter paper and evaporated to give the extract under vacuum. The extract was dissolved in water and this solution was extracted with ether three times. The water phase was separated and evaporated to dryness under vacuum. The water-soluble fraction was extracted with 1-butanol three times. The 1-butanol phase was separated and evaporated to dryness under vacuum.

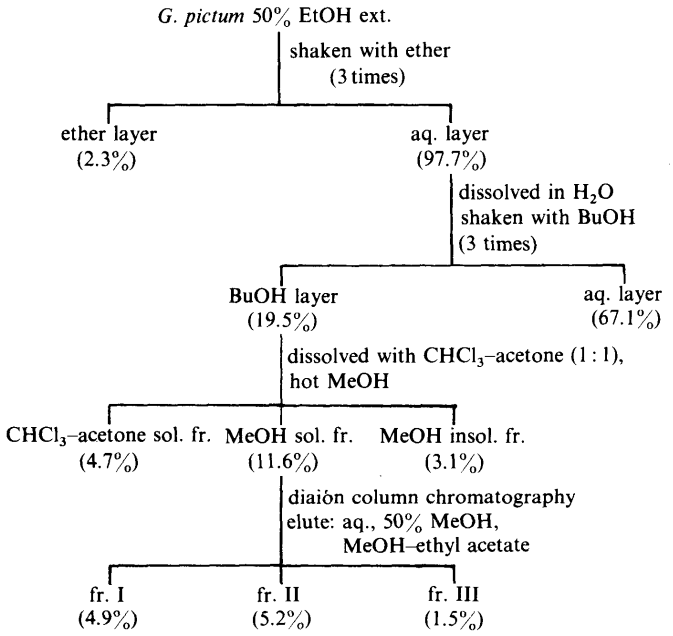

Fig. 1. Flow Diagram of Fractionation of the Ethanol Extract Obtained from *G. pictum*

Here (%) indicates percentage yield calculated on the basis of the methanol extract.

Then the 1-butanol-soluble fraction was extracted with chloroform–acetone (1 : 1), hot methanol and water, successively. The hot methanol-soluble fraction was separated and evaporated to dryness under vacuum; the residue thus obtained was chromatographed on Diaion HP-20, using water : ethyl acetate = 1 : 1, 50% methanol : ethyl acetate = 1 : 1 and methanol : ethyl acetate = 1 : 1 as the elution solvents to give three fractions (frs. I—III). Each fraction was evaporated to dryness under vacuum and assayed for its antiinflammatory effects. As shown in Fig. 1, the yields (%) were each suspended in 2% carboxymethyl cellulose (CMC) solution. The

The 50% ethanol extraction, each fraction, and indomethacin (Sigma) were each suspended in 2% canboxymethyl cellulose CMC solution. The dose for each of the fractions was chosen based on the yields obtained from the 50% ethanol extraction.

Carrageenin-Induced Hind-Paw Edema Test Male Wistar rats weighing 200—250 g were fasted for 16 h prior to experiments, but were supplied with water *ad libitum*. Carrageenin (Picnin-A, Zushikagaku Lab., Inc.) was suspended in saline to make a 1% (w/v) suspension. The suspension of carrageenin was injected subcutaneously into the right hind-paw 30 min after the test solutions had been administered orally.

The volume of the hind-paw was measured before the test solutions had been administered by a water displacement transducer (LPU-0.1A, Nihon Kohden). The hind-paw volumes were measured 30 min and 1 h after the suspension of carrageenin had been administered and then up to 6 h at intervals of 1 h.

Control rats were treated similarly, except that they received an oral dose of 2% CMC solution alone. The results were expressed as percent increase in hind-paw volume due to swelling, as compared with the initial hind-paw volume.

Acetic Acid-Induced Vascular Permeability Test Male ddY mice weighing 20—25 g were fasted for 2 h prior to experiments, but were supplied with water *ad libitum*. Four percent pontamine sky blue solution in saline (w/v) was injected intravenously into the tail vein 40 min after test solutions had been administered orally.

After 30 min, 1% acetic acid solution in saline (v/v) was injected intraperitoneally, and then after 20 min, mice were killed by dislocation of the neck, and the abdominal wall was cut to expose the entrails. After washing of the entrails with saline, the washings were filtered through glasswool and collected into test tubes. To clear any turbidity due to protein, 0.1 ml of 1 N NaOH solution was added to each tube, and the absorbance was read at 590 nm in a spectrophotometer (model 200-10, Hitachi). Control mice were treated similarly, except that they received an oral dose of 2% CMC solution alone.

The vascular permeability effects were expressed in terms of total dye (μg/animal) amount which leaked into the intraperitoneal cavity.

Acetic Acid-Induced Writhing Test Male ddY mice weighing 20—25 g were fasted for 2 h, but were supplied with water *ad libitum*. A 0.7%

solution of acetic acid in saline (v/v) was injected intraperitonealy 85 min after the test solutions had been administered orally. After 5 min, the number of writhes induced by the acetic acid solution was counted for 10 min.

Control mice were treated similarly, except that they received an oral dose of 2% CMC solution alone.

Statistical Analysis Data were expressed as the mean value ± standard error. All results were analyzed for variance by Bartlett's method and then significant differences were subsequently examined by Duncan's method.

Results

Effect of Ethanol Extract Obtained from *G. pictum* The ethanol extract (at 3 g/kg) showed a lasting inhibition of the edema induced by carrageenin during 6 h. The inhibitory potency was about the same as that of indomethacin (at 10 mg/kg) during 2 h and at 6 h, but was weaker than that of indomethacin at 3, 4 and 5 h, as shown in Fig. 2.

The ethanol extract (at doses of 1 and 3 g/kg) inhibited the increase of dye leakage induced by acetic acid. Indomethacin (at 10 mg/kg) inhibited the dye leakage with a potency about the same as that of the ethanol extract (at 3 g/kg), as shown in Table I.

The ethanol extract (at 3 g/kg) reduced the number of writhes induced by acetic acid. The inhibitory potency induced by the extract (at 3 g/kg) was about the same as that of indomethacin (10 mg/kg), as shown in Table II.

Effects of Each Fraction Obtained from the Ethanol Extract The water-soluble fraction (at 2.9 g/kg) showed an inhibition of edema during 30 min to 4 h after injection of carrageenin. On the other hand, the ether-soluble fraction (at 0.1 g/kg) did not significantly inhibit the edema. The inhibitory potency induced by the former fraction was about the same as that of indomethacin (at 10 mg/kg) during 4 h, but was weaker than that of indomethacin at 5

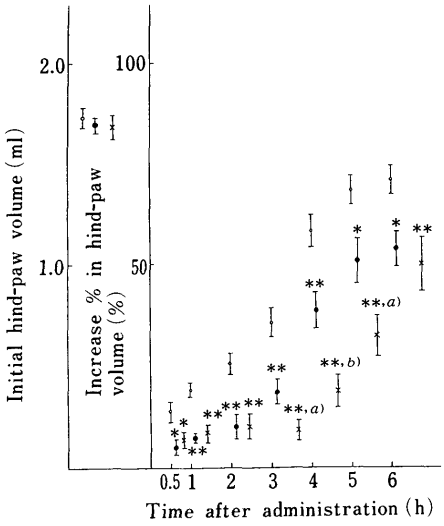

Fig. 2. Effect of the Ethanol Extract and Indomethacin on the Paw Edema Induced by Carrageenin in Rats

The results were expressed as percent increase in hind-paw volume due to swelling (%) (right column), as compared with the initial hind-paw volume (ml) before carrageenin injection (left column).

○, control (2% CMC) (*p.o.*) ($N=7$); ●, *G. pictum* 3 g/kg ($N=7$); ×, indomethacin 10 mg/kg ($N=7$).

*, ** Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *a*), *b*) Significantly different from *G. pictum* at $p<0.05$ and $p<0.01$, respectively.

TABLE I. Effect of the Ethanol Extract and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	Amount of leaked dye (μg/animal)
Control (2% CMC)		12	386.2 ± 22.8
G. pictum	1	6	307.0 ± 14.5[a]
	3	8	233.6 ± 24.1[b]
Indomethacin	0.01	8	233.8 ± 14.1[b,c]

a, *b*) Significantly different from the control $p<0.05$ and $p<0.01$, respectively. *c*) Not significantly different from *G. pictum* 3 g/kg.

TABLE II. Analgesic Effect of the Ethanol Extract and Indomethacin on the Writhing Symptom Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	No. of writhes (in 10 min)
Control (2% CMC)		10	39.2 ± 1.9
G. pictum	1	7	34.3 ± 1.9
	3	8	19.3 ± 3.5[a]
Indomethacin	0.01	9	22.3 ± 4.0[a,b]

a) Significantly different from the control at $p<0.01$. *b*) Not significantly different from *G. pictum* 3 g/kg.

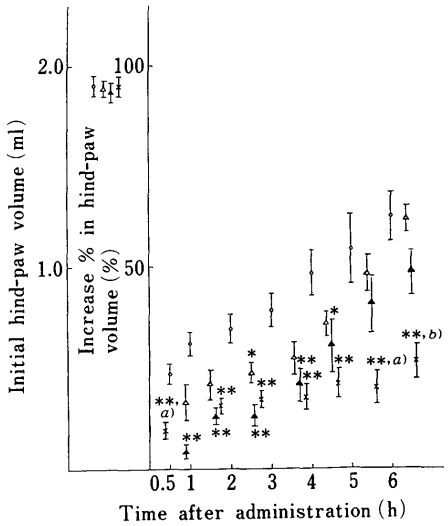

Fig. 3. Effect of the Ether-Soluble Fraction, the Water-Soluble Fraction and Indomethacin on the Paw Edema Induced by Carrageenin in Rats

The results were expressed as percent increase in hind-paw volume due to swelling (%) (right column), as compared with the initial hind-paw volume (ml) before carrageenin injection (left column).

○, control (2% CMC) (*p.o.*) ($N=10$); △, *G. pictum* ether layer 0.1 g/kg ($N=8$); ▲, aq. layer 2.9 g/kg ($N=8$); ×, indomethacin 10 mg/kg ($N=10$).

*, ** Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *a*), *b*) Significantly different from *G. pictum* aqueous layer at $p<0.05$ and $p<0.01$, respectively.

TABLE III. Effect of the Ether Soluble Fraction and the Water-Soluble Fraction Obtained from the Ethanol Extract and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	Amount of leaked dye (μg/animal)
Control (2% CMC)		10	392.5 ± 14.9
G. pictum ether layer	0.1	8	389.7 ± 13.2
G. pictum aq. layer	2.9	8	204.4 ± 29.5[a]
Indomethacin	0.01	10	205.4 ± 17.6[a,b]

a) Significantly different from the control at $p<0.01$. *b*) Not significantly different from the *G. pictum* aqueous layer.

and 6 h, as shown in Fig. 3.

The water-soluble fraction (at 2.9 g/kg) inhibited the increase of dye leakage induced by acetic acid, while the ether-soluble fraction (at 0.1 g/kg) did not. The inhibitory potency of the former was about the same as that of indomethacin (at 10 mg/kg), as shown in Table III.

The 1-butanol soluble fraction (at 0.7 g/kg) inhibited the increase of dye leakage induced by acetic acid. Its inhibitory potency was about the same as that of indomethacin (at 10 mg/kg), as shown in Table IV.

The hot methanol-soluble fraction (at 0.42 g/kg) inhibited the increase of dye leakage induced by acetic acid. But, its inhibitory potency was somewhat weaker than that of indomethacin (at 10 mg/kg), as shown in Table V.

As shown in Table VI, among these fractions obtained from the hot methanol fraction, only fr. II (at 0.19 g/kg) inhibited the increase of dye leakage. All of the other fractions were inactive. The inhibitory potency of fr. II was somewhat weaker than that of indomethacin (at 10 mg/kg).

Constituents of the Active Fraction As fr. II showed the inhibitory effect on the increase of dye leakage induced by acetic acid, the active constituents were studied by using high-performance liquid chromatography (HPLC) (Shimadzu, LC-3A). A reversed-phase column (Megapak SIL C_{18}) was eluted with a mixture of CH_3CN–H_2O (13 : 100), at a flow rate of 1 ml/min.

Fraction II showed several peaks on HPLC, and a methanol solution of fr. II showed a red-purple color on the reaction with magnesium ribbon and hydrochloric acid, indicating the presence of flavonoids.

TABLE IV. Effect of the 1-Butanol-Soluble Fraction, the Water-Soluble Fraction and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	Amount of leaked dye (μg/animal)
Control (2% CMC)		16	439.2 ± 19.2
G. pictum 1-BuOH layer	0.7	8	289.1 ± 40.8[a]
G. pictum aq. layer	2.2	8	439.1 ± 39.1
Indomethacin	0.01	14	274.9 ± 25.8[a,b]

a) Significantly different from the control at $p<0.01$. *b*) Not significantly different from the *G. pictum* 1-butanol layer.

TABLE V. Effect of the Chloroform–Acetone-Soluble Fraction, Hot Methanol-Soluble Fraction, Methanol-Insoluble Fraction and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	Amount of leaked dye (μg/animal)
Control (2% CMC)		9	428.9 ± 17.9
G. pictum $CHCl_3$–acetone sol. fr.	0.17	9	378.0 ± 19.5
G. pictum MeOH sol. fr.	0.42	9	302.2 ± 37.2[a]
G. pictum MeOH insol. fr.	0.11	9	432.0 ± 23.5
Indomethacin	0.01	9	273.1 ± 23.0[a,b]

a) Significantly different from the control at $p<0.01$. *b*) Not significantly different from the *G. pictum* methanol-soluble fraction.

TABLE VI. Effect of Fractions Obtained from Hot Methanol-Soluble Fraction and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	Amount of leaked dye (μg/animal)
Control (2% CMC)		9	366.6 ± 34.3
G. pictum fr. I	0.18	9	382.7 ± 43.3
G. pictum fr. II	0.19	9	260.0 ± 20.7[a]
G. pictum fr. III	0.05	9	356.5 ± 38.7
Indomethacin	0.01	9	240.6 ± 17.8[a,b]

a) Significantly different from the control at $p<0.05$. *b*) Not significantly different from the *G. pictum* fraction II.

Discussion

In the present study, it was found that the 50% ethanol extract obtained from red leaves of *G. pictum*, which is used in Indonesian folk medicine as mentioned above, inhibited the edema induced by carrageenin and the increase of dye leakage induced by acetic acid, and it also reduced the writhing induced by acetic acid. From these results, it seems likely that the extract of *G. pictum* has antiinflammatory and analgesic effects. Therefore, it was considered worthwhile to elucidate the antiinflammatory activity of the extract and to study the active principles in it.

The water-soluble fraction obtained from the ethanol extract inhibited the edema induced by carrageenin and the increase of dye leakage induced by acetic acid, but the ether-soluble fraction did not. As the antiinflammatory activity had been concentrated in the water-soluble fraction, this fraction was further fractionated, based on the results of antiinflammatory activity assay using the experimental model of dye leakage. Among these fractions, the activity shifted to the 1-butanol-soluble fraction, hot methanol fraction and fr. II, successively. As the potency of the inhibitory effects induced by these fractions was approximately the same as that of a fixed dose of indomethacin in all experiments, it is considered that the antiinflammatory activity had been obtained almost entirely in fr. II.

The methanol solution of fr. II showed a red-purple color on reaction with magnesium ribbon and hydrochloric acid, suggesting that fr. II contained flavonoids. Many kinds of flavonoid compounds are widely distributed in many plants and there have been numerous reports on the antiinflammatory effects of flavonoid compounds.[5-10] From these results it is suggested that these flavonoids are at least partly responsible for exerting the antiinflammatory effect of the ethanol extract of the red leaves of *G. pictum*.

It is well known that the development of edema induced by carrageenin and the increase of vascular permeability induced by acetic acid correspond to the early exudative stage of inflammation, one of the important processes of inflammatory pathology.[11,12] *G. pictum* inhibited the edema induced by carrageenin and the increase of the vascular permeability induced by acetic acid in the present study, which shows that it exerts an antiinflammatory effect, at least at the early exudative stage of inflammation.

Brown *et al.* reported that centrally acting drugs inhibited the edema induced by carrageenin in the hind paw of the rat.[13] However, each fraction, at the doses used in this experiment, was found to be free from any apparent effect on the central nervous system in mice and rats, and fr. II may contain polar compounds rather than nonpolar compounds. These results suggest that the antiinflammatory effects of *G. pictum* may not be exerted through the

central nervous system.

The red leaves of *G. pictum* are used in Indonesian folk medicine as a poultice on cuts, wounds and all kinds of swellings. On the basis of these uses, we examined the extract and found that it dose have antiinflammatory effects. The findings of this study thus lend support to the traditional uses of this plant in the treatment of some inflammations.

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