Antiinflammatory Effect of *Curcuma xanthorrhiza* ROXB. and Its Active Principles

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The rhizomes of *Curcuma xanthorrhiza* ROXB. are used in Indonesian folk medicine as cholagogues, aromatic stomachics, analgesics, a rheumatic remedy, *etc.* The present study was carried out to elucidate the antiinflammatory effect of the methanol extract obtained from these rhizomes and its active principles. The methanol extract was partitioned between ether and water, and then the ether-soluble fraction was extracted with *n*-hexane. The *n*-hexane-soluble fraction was chromatographed (fr. I—IV), fr. II was rechromatographed (fr. V—VII), and then fr. V was rechromatographed (fr. VIII—X) by silica gel column chromatography. 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 methanol extract (*p.o.*) showed both an antiinflammatory activity and an analgesic activity and these activities shifted successively to the ether-soluble fraction, the *n*-hexane-soluble fraction, fr. II, V and IX. The chemical structure of fr. IX was identified as germacrone. These results suggest that the antiinflammatory action of *Curcuma xanthorrhiza* is the result of the germacrone that it contains.

Keywords antiinflammatory effect; carrageenin-induced edema; vascular permeability; *Curcuma xanthorrhiza*; germacrone

The rhizomes of *Curcuma xanthorrhiza* ROXB. (*C. xanthorrhiza*) are used in Indonesian folk medicine as cholagogues, aromatic stomachics, analgesics and a rheumatic medicine.[1-5] We previously reported that the essential oil obtained by steam distillation from the rhizomes of *C. xanthorrhiza* caused a lasting increase of bile secretion and produced a rise in the excreted amount of total bile acids when orally administered to anesthetized rats, and that the cholagogic effect of the essential oil was attributable to the *d*-camphor it contains.[6]

Although many pharmacological studies have been reported,[7-9] there have been very few antiinflammatory studies on this subject.

On the basis of these uses in folk medicine, the present study was carried out to elucidate the antiinflammatory effect of 70% methanol extract obtained from the rhizomes of *C. xanthorrhiza* and to identify the active principle(s).

Experimental

Materials Fresh rhizomes of *C. xanthorrhiza* were cultivated in the Bandung region and refluxed with 70% methanol three times for 6 h each time. The solution was filtered through filter paper and evaporated to give the extract under vacuum.

The extract was dissolved in ether and extracted with water three times. The ether phase was separated and evaporated to dryness under vacuum. The ether-soluble fraction was then dissolved in *n*-hexane and extracted with methanol three times. The *n*-hexane phase was separated and evaporated to dryness under vacuum.

The *n*-hexane-soluble fraction was chromatographed into fractions I—IV (frs. I—IV) on a silica gel column, using an elution solvent of *n*-hexane, benzene, ethyl acetate and methanol. Fraction II was rechromatographed into frs. V—VII on a silica gel column, using an elution solvent of *n*-hexane, benzene and ethyl acetate.

As fr. V showed three spots on thin layer chromatography (Kieselgel 60 F_{254}, Merck), fr. V was rechromatographed into frs. VIII—X on silica gel column, using an elution solvent of benzene and chloroform, to monitor these spots. The solvent system used was benzene–chloroform (1 : 3), and spots on the plate were detected under ultraviolet (UV) light. Fraction IX showed only a large spot on the thin layer plate (Rf=0.49), and it gave a crystal as colorless needles, mp 52—54 °C. The chemical structure of this compound was identified as germacrone by comparison of UV, infrared (IR), nuclear magnetic resonance (NMR) and gas chromatography-mass spectra (GC-MS) with germacrone.

The methanol extract and each fraction were assayed for antiinflammatory effects. As shown in Fig. 1, the yields (%) were calculated on the basis of the methanol extract.

The methanol extract, each fraction, and indomethacin (Sigma) were each suspended in 2% carboxymethylcellulose (CMC) solution. The dose for each of the fractions was chosen based on the yields obtained from the 70% methanol 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.)

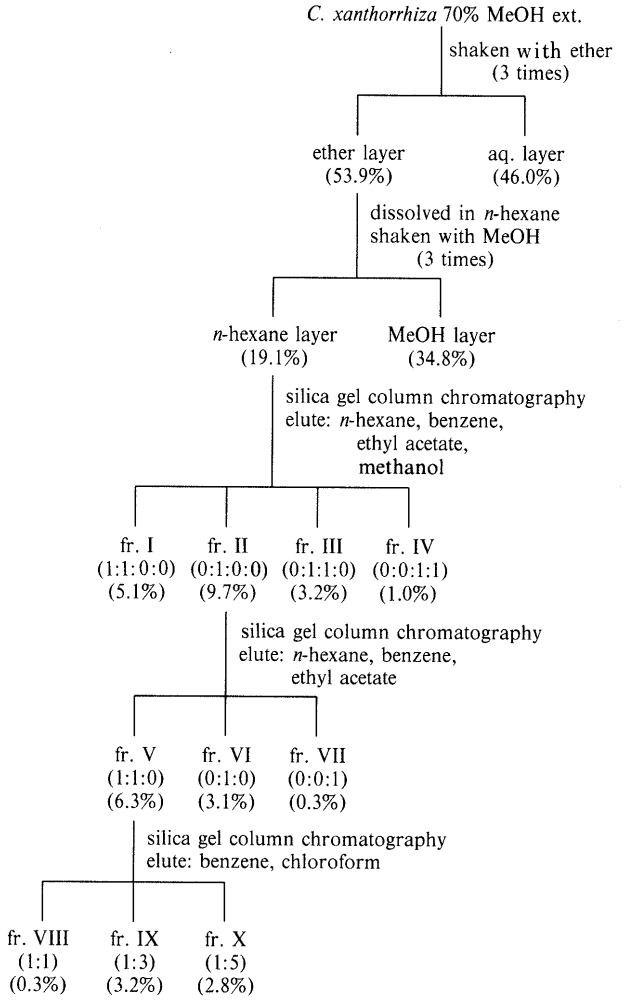

Fig. 1. Flow Diagram of Fractionation of the Methanol Extract Obtained from *C. xanthorrhiza*

Here, (%) indicates percentage yield calculated on the basis of the methanol extract obtained from *C. xanthorrhiza*.

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 prior to administration of the test solutions 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 at intervals of 1 h for up to 6 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 the oral administration of test solutions.

After 30 min, 1% acetic acid solution in saline (v/v) was injected intraperitoneally, and 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 glass wool and collected in 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 the amount of total dye (μg/animal) 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 intraperitoneally 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 Methanol Extract Obtained from *C. xanthorrhiza* The mthanol extract (at 3 g/kg) showed a lasting inhibition of the edema induced by carrageenin during the 6 h period. The inhibitory potency was about the same as that of indomethacin (at 10 mg/kg) (Fig. 2). In the preliminary experiment, the lower dose of methanol extract

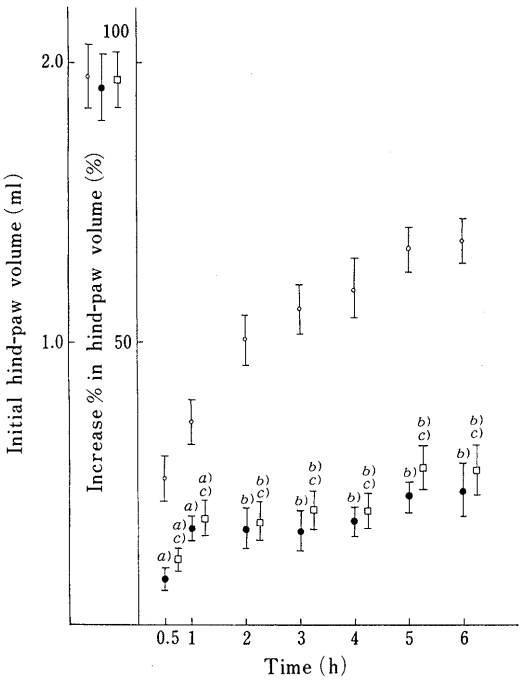

Fig. 2. Effect of the Methanol Extract and Indomethacin on the Paw Edema Induced by Carrageenin in Rats

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.*) ($n=7$); ●, *C. xanthorrhiza* 3.0 g/kg ($n=7$); □, indomethacin 10 mg/kg ($n=7$). *a, b*) Significantly different from the control at $p<0.01$ and $p<0.001$, respectively. *c*) Not significantly different from *C. xanthorrhiza* 3 g/kg.

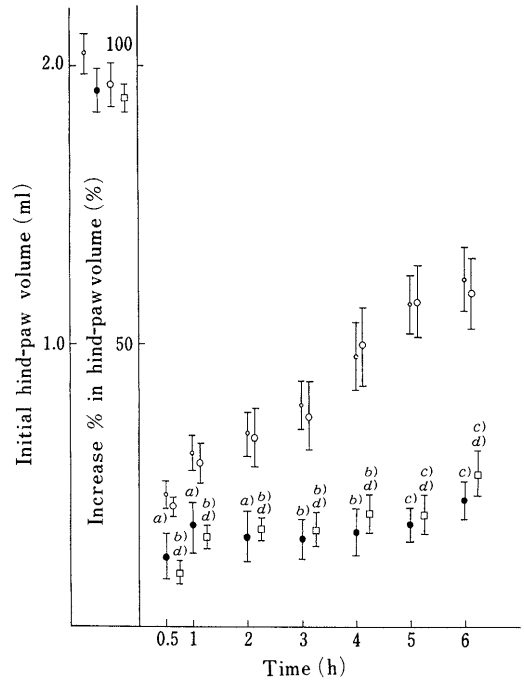

Fig. 3. Effect of the Ether-Soluble Fraction, the Water-Soluble Fraction and Indomethacin on the Paw Edema Induced by Carrageenin in Rats

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.*) ($n=11$); ●, *C. xanthorrhiza* ether layer 1.4 g/kg ($n=7$); ○, *C. xanthorrhiza* aq. layer 1.6 g/kg ($n=7$); □, indomethacin 10 mg/kg ($n=11$). *a, b, c*) Significantly different from the control at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. *d*) Not significantly different from *C. xanthorrhiza* ether layer 1.4 g/kg.

TABLE I. Effect of the Methanol 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	395.2 ± 37.1
C. xanthorrhiza	1	8	294.2 ± 10.7[a]
	3	8	218.4 ± 15.9[b]
Indomethacin	0.01	8	219.8 ± 26.3[b,c]

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

TABLE II. Analgesic Effect of the Methanol 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
C. xanthorrhiza	1	7	25.7 ± 5.8
	3	8	17.8 ± 4.9[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 *C. xanthorrhiza* 3 g/kg.

Table III. Effect of the Ether-Soluble Fraction and 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)		10	392.5±14.9
C. xanthorrhiza			
ether layer	1.4	8	196.2±19.5[a]
aq. layer	1.6	8	378.9±26.8
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 *C. xanthorrhiza* ether layer 1.4 g/kg.

Table IV. Effect of the *n*-Hexane-Soluble Fraction and the 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)		16	439.2±19.2
C. xanthorrhiza			
n-hexane layer	0.5	8	235.6±33.1[a]
MeOH layer	0.9	8	340.9±31.5[b]
Indomethacin	0.01	14	274.9±25.8[a,c]

a, b) Significantly different from the control $p<0.01$ and $p<0.05$, respectively. *c*) Not significantly different from *C. xanthorrhiza n*-hexane layer 0.5 g/kg.

(at 1 g/kg) did not show a significant inhibitory effect on the edema (data not shown in the figure).

The methanol extract (at doses of 1 and 3 g/kg) inhibited dose-dependently 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 methanol extract (at 3 g/kg) (Table I).

The 1 and 3 g/kg doses of methanol extract reduced dose-dependently 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 (at 10 mg/kg) (Table II).

Effects of Each Fraction Obtained from the Methanol Extract The ether-soluble fraction obtained from the methanol extract (at 1.4 g/kg) showed a lasting inhibition of edema for from 30 min to 6 h after injection of carrageenin. On the other hand, the water-soluble fraction (at 1.6 g/kg) did not inhibit the edema. The inhibitory potency induced by the ether-soluble fraction was about the same as that of indomethacin (at 10 mg/kg) (Fig. 3).

The ether-soluble fraction (at 1.4 g/kg) inhibited the increase of dye leakage induced by acetic acid, while the water-soluble fraction (at 1.6 g/kg) did not. The inhibitory potency induced by the ether soluble fraction was about the same as that of indomethacin (at 10 mg/kg) (Table III).

The *n*-hexane-soluble fraction obtained from the ether-soluble fraction (at 0.5 g/kg) and the methanol-soluble fraction (at 0.9 g/kg) inhibited the increase of dye leakage induced by acetic acid. The inhibitory potency induced by the *n*-hexane-soluble fraction was stronger than that of the methanol-soluble fraction and was about the same as that of indomethacin (at 10 mg/kg) (Table IV). From these results, it was suggested that the pharmacologically active principle(s) passed mainly into the *n*-hexane-soluble fraction.

Table V. Effect of Fractions Obtained from the *n*-Hexane-Soluble Fraction and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

Compound	Dose (g/kg *p.o.*)	No. of animals	No. of leaked dye (μg/animal)
Control (2% CMC)		8	348.2±18.4
C. xanthorrhiza			
fr. I	0.13	8	323.9±32.9
fr. II	0.26	8	189.6±19.4[a]
fr. III	0.08	8	318.2±26.2
fr. IV	0.03	8	358.9±26.0
Indomethacin	0.01	8	239.8± 7.4[a,b]
Control (2% CMC)		8	414.6±22.1
C. xanthorrhiza			
fr. V	0.17	8	182.0±22.4[a]
fr. VI	0.08	8	351.6±21.4
fr. VII	0.01	8	360.2±22.9
Indomethacin	0.01	8	251.2±24.5[a,c]
Control (2% CMC)		8	385.7±12.2
C. xanthorrhiza			
fr. VIII	0.006	8	333.2±26.0
fr. IX	0.075	8	242.8±21.8[a]
fr. X	0.067	8	354.9±12.2
Indomethacin	0.01	8	259.4±21.2[a,d]

a) Significantly different from the control at $p<0.01$ in each experiment. *b, c* and *d*) Not significantly different from fr. II 0.26 g/kg, fr. V 0.17 g/kg and fr. IX 0.075 g/kg in each experiment, respectively.

Table VI. Effect of Fractions Obtained from Fraction V 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)		7	39.6±2.5
C. xanthorrhiza			
fr. VIII	0.006	7	36.0±2.1
fr. IX	0.075	7	16.6±4.4[a]
fr. X	0.065	7	34.0±2.5
Indomethacin	0.01	7	19.1±3.5[a,b]

a) Significantly different from the control at $p<0.01$. *b*) Not significantly different from fr. IX 0.075 g/kg.

As shown in Table V, among the fractions obtained from the *n*-hexane-soluble fraction, only fr. II (at 0.26 g/kg), fr. V (at 0.17 g/kg) and fr. IX (at 0.075 g/kg) inhibited the increase of dye leakage induced by acetic acid; none of the others significantly inhibited the increase. The inhibitory potency of frs. II, V and IX was about the same as that of indomethacin (at 10 mg/kg), respectively.

Fraction IX (at 0.075 g/kg) also reduced the number of writhes induced by acetic acid; none of the other fractions did so significantly. The inhibitory potency induced by fr. IX was about the same as that of indomethacin (at 10 mg/kg) (Table VI).

Discussion

In the present study, it was found that the 70% methanol extract obtained from *C. xanthorrhiza* inhibited significantly the edema induced by carrageenin, the increase of the dye leakage induced by acetic acid and the number of writhes induced by acetic acid at the same respective dose of 3 g/kg, and that the inhibitory potency induced by the extract was about the same as that of indomethacin at

10 mg/kg.

It is well known that irritating compounds sometimes cause pseudo inhibition of the edema induced by carrageenin. But the methanol extract inhibited the increase of the dye leakage induced by acetic acid and the number of writhes induced by acetic acid.

From these results, it seems likely that the methanol extract does not have an irritating effect and shows both antiinflammatory and analgesic effects. Therefore, it was considered worthwhile to elucidate the antiinflammatory activity of the extract and to isolate the active principles from it.

The ether-soluble fraction obtained from the methanol extract inhibited the edema induced by carrageenin and the increase of the dye leakage induced by acetic acid, but the water-soluble fraction did not. As the antiinflammatory activity had been concentrated in the ether-soluble fraction, this was further fractionated, based on the results of antiinflammatory activity assay using the experimental mode of dye leakage induced by acetic acid.

The activity shifted successively to the *n*-hexane-soluble fraction, frs. II and V. Since 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 almost entirely in fr. V. Then, the active principle (fr. IX) was isolated from fr. V and its chemical structure was identified as germacrone.

Germacrone (fr. IX) obtained from fr. V inhibited the increase of the dye leakage induced by acetic acid and also reduced the number of writhes induced by acetic acid.

These results suggest that the antiinflammatory effect of the methanol extract is due to its germacrone content and also that germacrone may exert an analgesic effect.

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.[10,11] Germacrone 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 an early exudative stage of inflammation.

Yamazaki *et al.* recently reported that the methanol extract of rhizomes of *C. xanthorrhiza* on oral administration showed a hypothermic effect and prolongation of pentobarbital-induced sleeping time.[8] They also isolated germacrone from the extract and reported that, at doses of 100 and 200 mg/kg, the compound suppressed spontaneous motor activity, acetic acid-induced writhing and showed no lethal toxicity at 750 mg/kg when orally administered to mice.[9] From these results, they concluded that the depressive effects of methanol extract obtained from *C. xanthorrhiza* on the central nervous system might be mainly due to the germacrone that it contained. Brown *et al.* then reported that many centrally acting drugs inhibited the edema induced by carrageenin in the hind-paw of rats.[12]

Although neither fractions nor the germacrone (at 75 mg/kg) at the doses used in this experiment, was found to have any apparent effect on the central nervous system or on toxicity in mice and rats, these reports suggest that the antiinflammatory effects of germacrone may be partly exerted through the central nervous system.

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References

1) H. H. Hirshhorn, *J. Ethnopharm.*, **7**, 123 (1983).
2) Y. S. Kasahara and S. Mangunkawatja, "Medicinal Herb Index in Indonesia," P. T. Eisai, Indonesia, 1986, p. 343.
3) S. Elliott and J. Brimacombe, *J. Ethnopharm.*, **19**, 285 (1987).
4) M. L. Perry, "Medicinal Plants of East and South-East Asia; Attributed Properties and Uses," MIT Press, Cambridge, United States and London, 1980, p. 440.
5) H.-Y. Hsu, Y.-P. Chen, S.-J. Shen, C.-S. Hsu, C.-C. Chen and H.-C. Chang, "Oriental Materia Medica; A Concise Guide," Oriental Healing Arts Institute, U.S.A., 1986, p. 451.
6) Y. Ozaki and O. B. Liang, *Shoyakugaku Zasshi*, **42**, 257 (1988) (in English).
7) Y. Ozaki and S. Soedigdo, *Shoyakugaku Zasshi*, **42**, 333 (1988) (in Japanese).
8) M. Yamazaki, Y. Maebayashi, N. Iwase and T. Kaneko, *Chem. Pharm. Bull.*, **36**, 2070 (1988).
9) M. Yamazaki, Y. Maebayashi, N. Iwase and T. Kaneko, *Chem. Pharm. Bull.*, **36**, 2075 (1988).
10) A. C. Winter, A. E. Risley and W. G. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
11) A. B. Whittle, *Br. J. Pharmacol.*, **22**, 246 (1964).
12) H. J. Brown, W. J. Kissel and M. P. Lish, *J. Pharmacol. Exp. Ther.*, **160**, 231 (1968).