ORIGINAL ARTICLE

Investigation the antinociceptive, antipyretic and anti-inflammatory activities of *Curcuma aeruginosa* Roxb. extracts in experimental animals

Wantana Reanmongkol[1], Sanan Subhadhirasakul[2], Narinee Khaisombat[3], Parichat Fuengnawakit[3], Saranya Jantasila[3] and Arunee Khamjun[3]

Abstract

Reanmongkol, W., Subhadhirasakul, S., Khaisombat, N., Fuengnawakit, P., Jantasila, S. and Khamjun, A.

Investigation the antinociceptive, antipyretic and anti-inflammatory activities of *Curcuma aeruginosa* Roxb. extracts in experimental animals

Songklanakarin J. Sci. Technol., 2006, 28(5) : 999-1008

***Curcuma aeruginosa* (*C. aeruginosa*) Roxb. (Zingiberaceae) is known in Thai as Waan-Ma-Haa-Mek. The rhizomes of this plant have been used as a component of Thai herbal medicinal recipes used for decreasing dysmenorrhea. In the present study, the analgesic, antipyretic and anti-inflammatory actions of this plant were investigated in experimental animals. The rhizomes of *C. aeruginosa* were extracted with chloroform, methanol and water to give chloroform, methanol and aqueous extracts, respectively. The effects of the three extracts on nociceptive response using writhing, hot plate and formalin tests in mice were performed. The antipyretic activity in yeast-induced fever and the anti-inflammatory activity in carrageenin-induced edema in rats, were examined. The LD_{50} value of orally administered the chloroform extract and methanol extract**

[1]Ph.D.(Pharmacology), Assoc. Prof., Department of Clinical Pharmacy, [2]Ph.D.(Chemistry of Natural Products), Assoc. Prof., Department of Pharmacognosy and Pharmaceutical Botany, [3]Pharmacy students, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand.

Corresponding e-mail: wantana.r@psu.ac.th

Received, 20 September 2005 Accepted, 28 March 2006

in mice was 3.03 g/kg. No dead mice were observed after oral administration of aqueous extract at the dose of 10 g/kg. Oral administration of the chloroform extract and the methanol extract of *C. aeruginosa* rhizomes (100-400 mg/kg) significantly decreased the number of writhings and stretchings induced by acetic acid. Only the chloroform extract suppressed the licking activity of the late phase in the formalin test in mice. All extracts of *C. aeruginosa* rhizomes had no effects on heat-induced pain in mice, yeast-induced fever and carrageenin-induced edema in rats. These results suggest that the chloroform extract of *C. aeruginosa* rhizome possesses analgesic effect via a different mechanism from that of the aspirin.

Key words : *Curcuma aeruginosa*, antinociceptive, antipyretic, anti-inflammatory

บทคัดย่อ

วันทนา เหรียญมงคล[1] สนั่น ศุภธีรสกุล[2] นาริณี ไข่สมบัติ[1] ปาริฉัตร เฟื่องนวกิจ[1]
สรัญญา จันทะสิละ[1] และ อรุณี คำจุ่น[1]

ศึกษาฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดว่านมหาเมฆในหนูทดลอง

ว. สงขลานครินทร์ วทท. 2549 28(5) : 999-1008

Curcuma aeruginosa **มีชื่อเรียกในภาษาไทยว่า ว่านมหาเมฆ มีการนำเหง้าของพืชนี้มาใช้เป็นยาในตำรับยาสมุนไพรไทยสำหรับลดอาการปวดประจำเดือน ในการศึกษานี้ ได้ทำการทดสอบฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของพืชนี้ในสัตว์ทดลอง สกัดเหง้าของว่านมหาเมฆด้วยคลอโรฟอร์ม เมทานอล และน้ำ ตามลำดับ นำสารสกัดที่ได้มาทดสอบฤทธิ์ระงับปวดที่เหนี่ยวนำโดยกรดอะเซติก ความร้อน และฟอร์มาลินในหนูถีบจักร รวมทั้งฤทธิ์ลดไข้ที่เหนี่ยวนำโดยยีสต์ในหนูขาว และผลลดการบวมที่อุ้งเท้าที่เหนี่ยวนำด้วยคาร์ราจีนินในหนูขาว ขนาดของสารสกัดด้วยคลอโรฟอร์ม และสารสกัดด้วยเมทานอล ที่ทำให้หนูถีบจักรตาย 50% เมื่อป้อนทางปาก มีค่าเท่ากันคือ 3.03 กรัม/กก. ไม่มีหนูถีบจักรตายเมื่อป้อนสารสกัดด้วยน้ำทางปากในขนาดสูง 10 กรัม/กก. การป้อนสารสกัดด้วยคลอโรฟอร์มและเมทานอลจากเหง้าของว่านมหาเมฆ (100-400 มก./กก.) ทางปากในหนูถีบจักร สามารถลดจำนวนของการบิดและยืดของลำตัวเมื่อถูกกระตุ้นโดยกรดอะเซติก เฉพาะสารสกัดด้วยคลอโรฟอร์ม สามารถลดการเลียในช่วงเฟสหลังของการทดสอบด้วยฟอร์มาลินในหนูถีบจักร ไม่มีสารสกัดใดจากเหง้าของว่านมหาเมฆ มีผลต่อความเจ็บปวดที่เกิดจากความร้อนในหนูถีบจักร อาการไข้ที่เหนี่ยวนำด้วยยีสต์ และการบวมของอุ้งเท้าที่เหนี่ยวนำด้วยคาร์ราจีนินในหนูขาว จากผลการทดลองนี้เสนอว่า สารสกัดด้วยคลอโรฟอร์มจากเหง้าของว่านมหาเมฆ มีฤทธิ์ระงับปวด โดยมีกลไกการออกฤทธิ์ที่แตกต่างจากแอสไพริน**

[1]ภาควิชาเภสัชกรรมคลินิก [2]ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Curcuma aeruginosa (*C. aeruginosa*) Roxb. (Zingiberaceae) is known in Thai as Waan-Ma-Haa-Mek. The rhizome of this plant is used medicinally to treat asthma and cough, scurvy and mental derangements (Perry, 1980). It also added in a beverage given to women in confinement to accelerate the lochia and decrease pain and inflammation of uterus (Perry, 1980; Pongbunrod, 1979). It is considered to be depurative and used both internally and externally for treating exanthema and also as a poultice for itching (Perry, 1980).

Some chemical constituents and biological activities of *C. aeruginosa* have been reported. Zedoalactone A, zedoalactone B, isofuranodiene, furanodiene, furanodienone, dehydrocurdione, curcumenone, 13-hydroxygermacrone and zedoarol, sesquiterpenes and guaianolide zedoarondiol have been isolated from the rhizomes of *C. aeruginosa* (Takano *et al.*, 1995; Sirat *et al.*,

1998a). The essential oils of the rhizomes and leaves of *C. aeruginosa* have been identified as 1,8-cineole, curzerenone, furanogermenone, camphor, (Z)-3-hexenol, zedoarol, furanodienone, curcumenol, isocurcumenol, beta-elemene, curzerene and germacrone (Xuan Dung *et al*., 1995; Sirat *et al*.,1998b; Bin Jantan *et al*.,1999; Jirovetz *et al*., 2000). The water extract from the rhizome of *C. aeruginosa* effectively inhibited on HIV-1-infected MT-4 cells (Otake *et al*., 1995). Recently, it has been reported that the methanol extract of this plant showed an inhibitory effect on platelet-activating factor receptor binding to rabbit platelets (Jantan *et al*., 2005).

Although *C. aeruginosa*, especially rhizomes, has been used as herbal medicine in Thailand as a composition in Thai medicinal recipes used for decreasing dysmenorrhea, no pharmacological studies *in vivo* have previously been conducted on analgesic, antipyretic and anti-inflammatory actions of this plant. In the present study, we evaluated the potential existence of analgesic, antipyretic and anti-inflammatory activities of the extracts obtained from *C. aeruginosa* rhizomes using the writhing, hot plate and formalin tests in mice, antipyretic activity in yeast-induced fever and anti-inflammatory activity in carrageenin-induced paw edema in rats.

Material and Methods

Plant material

Fresh rhizomes of *Curcuma aeruginosa* (*C. aeruginosa*) Roxb. were collected in February, 2003 from Songkhla Province, Thailand. The voucher specimen (number : SN 4601010) was identified by Assoc. Prof. Dr. Sanan Subhadhirasakul, and was kept at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Preparation of the extracts from the rhizomes of *Curcuma aeruginosa*

Briefly, chopped-dried rhizomes of *C. aeruginosa* (1.9 kg) were extracted with $CHCl_3$ (10 L) for 7 days at room temperature and filtered; the maceration procedure was repeated 4 times. The solvent was evaporated under reduced pressure to afford the chloroform extract (81.5 g). The marc was dried at room temperature overnight and subsequently extracted with MeOH by the same procedure as mentioned above to afford MeOH extract (122.1 g). Other chopped-dried rhizomes of *C. aeruginosa* (3.5 kg) were extracted with boiling distilled water (15 L) for 1 hour. After filtration, the marc was reextracted twice by the same procedure. The solvent was evaporated under reduced pressure to afford the water extract (247.4 g).

Animals

All animals used in this study were obtained from the Animal House, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Male Swiss mice and Wistar rats weighing 30-38 g and 150-220 g, respectively, were used. The animals were housed for at least one week and trained to get used to handling for 5-10 min daily prior to testing. Food and water were given *ad libitum* unless otherwise specified. All animal experiments were approved by the Institutional Committee for Ethical Use of Animals, Prince of Songkla University, Thailand.

Acute toxicity

The 50% lethal dose of the methanol, chloroform and aqueous extracts of *C. aeruginosa* was estimated by the up-and-down method in mice (Bruce, 1985). Doses were adjusted by a constant multiplicative factor; viz. 1.5, for this experiment. The dose for each successive animal was adjusted up or down depending on the previous outcome.

Antinociceptive Activity

1. Writhing test

Writhing behaviour was tested in mice, in which 0.6% acetic acid solution (10 ml/kg body weight) was injected intraperitoneally and the number of writhings and stretchings was counted over a 20-min period as previously reported (Koster *et al*., 1959; Hendershot & Forsaith, 1959). Each type of plant extract (100, 200 and 400 mg/

kg), a reference analgesic drug, aspirin (200 mg/kg), or cosolvent vehicle was orally administered 30 min before acetic acid.

2. Hot plate test

The hot plate test was carried out according to the method described by Woolfe & MacDonald (1944). Mice were placed on a hot plate maintained at 55ºC ± 1ºC. Latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured. Thirty minutes after p.o. administration of the test agents, or 15 min after s.c. administration of morphine, the nociceptive response was measured every 15 min over a 60 min period. The cut-off time was 45 sec. Only the mice that showed nociceptive responses within 15 sec were used for the experiments.

3. Formalin test

Thirty minutes after administration of each extract of *C. aeruginosa* (100, 200 and 400 mg/kg, p.o.), aspirin (200 mg/kg, p.o.) or cosolvent or 15 min after s.c. administration of morphine 10 mg/kg), 20 µl of 2.5% formalin in saline was injected subcutaneously in a hind paw of the mice. The time spent for licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection (Hunskaar *et al*., 1985).

Antipyretic activity

Antipyretic actvity of drug was measured by slightly modifying the method described by Adams *et al*. (1968). Male Wistar rats were fasted overnight with water *ad lib* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the animals' dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showing an increase in temperature of at least 0.7ºC were used for the experiments. Test agent or cosolvent vehicle was administered orally and the temperature was measured at 1, 2, 3, 4 and 5 hr after drug administration.

Carrageenin-induced paw edema

According to the method described by Winter *et al*. (1962), the initial right hind paw volume of the rats was measured using a plethysmometer (Ugo Basile) and then 0.1 ml of 1% (w/v) carrageenin was subcutaneously injected into the subplantar region of the right hind paw. The volume of the paw was measured at 1, 2, 3, 4, and 5 hr after carrageenin injection, and the edema volume was determined. The data were expressed as paw volume (ml), compared with the initial hind paw volume of each rat. Test agents were orally administered 30 min before carrageenin injection.

Chemicals

The following drugs and AR grade of chemicals were used: morphine sulfate, brewer's yeast, carrageenin lambda (Sigma Chem. Co., St. Louis, U.S.A.), aspirin and tween 80 (Srichand United Dispensary Co., Ltd., Bangkok, Thailand), sodium chloride (Carlo Erba, Germanny), acetic acid (AR grade, J.T, Baker Inc., Phillipsburg, U.S.A.), propylene glycol (Vidhyasom Co., Ltd., Bangkok, Thailand), chloroform and methanol (AR grade, Merck KGaA, Germany). The extracts of *C. aeruginosa* rhizomes and aspirin were dissolved in cosolvent solution (propylene glycol : tween 80 : distilled water = 4:1:4), and administered orally in a constant volume of 10 ml/kg for mice and 5 ml/kg for rats. Morphine sulfate was dissolved in 0.9% sodium chloride solution and administered subcutaneously. All drug solutions were prepared immediately before using.

Statistical Analysis

Data are expressed as mean±SEM and were analyzed statistically by one-way ANOVA procedures, followed by Dunnett's test. A difference was considered significant at $p<0.05$.

Results

Acute Toxicity

In the acute toxicity test, signs of toxicity including muscle weakness, ataxia, lethargy, loss

of righting reflex, dyspnea and death were observed. The LD_{50} value of the chloroform extract and methanol extract of *C. aeruginosa* in mice was 3.03 g/kg. No dead mice were observed after oral administration of the aqueous extract at the dose of 10 g/kg.

Table 1. Effect of the chloroform extract, methanol extract and water extract from *C. aeruginosa* and aspirin on acetic acid-induced writhing response in mice.

No. of writhing (counts/20 min)					
Cosolvent (control)	Aspirin 200 mg/kg p.o.	*C. aeruginosa* extract	Dose (mg/kg p.o.)		
			100	200	400
33.6±5.1	9.6±2.6*	Chloroform ext.	35.2±5.8	21.9±5.2	10.7±3.8*
48.1±4.4	12.7±3.3*	Methanol ext.	32.8±5.3	28.2±5.5*	24.6±3.7*
62.1±5.9	36.2±7.1*	Water ext.	62.7±3.9	57.0±9.7	64.7±4.4

The chloroform extract, methanol extract and water extract from *C. aeruginosa* were orally administered. After 30 min, 0.6% acetic acid solution (10 ml/kg) was intraperitoneally injected in mice. Immediately after injection, the number of writhings was counted over a 20-min period. Each datum represents the mean ± S.E.M. from 10 mice. * p< 0.05 compared with the control group (Dunnett's test).

Table 2. Effect of the chloroform extract, methanol extract and water extract from *C. aeruginosa*, aspirin and morphine on nociceptive response induced by heat in mice.

Drug	Dose (mg/kg) p.o.	Latency of nociceptive response (sec)					
		T_b min	0 min	15 min	30 min	45 min	60 min
Cosolvent	-	11.75±0.56	10.99±±0.85	11.44±1.06	11.79±0.92	13.38±2.35	12.90±1.40
Aspirin	200	11.03±0.76	12.41±1.33	13.57±1.59	13.54±1.35	10.37±0.79	10.72±1.17
C. aeruginosa	100	11.66±0.59	12.53±1.50	13.26±1.15	13.06±0.95	11.40±0.83	11.22±1.04
(chloroform	200	11.22±0.49	11.75±1.15	11.56±1.33	11.00±1.88	10.14±0.95	10.80±1.18
extract)	400	12.86±0.31	12.88±1.00	12.24±0.93	13.30±1.09	14.45±2.26	12.10±0.80
Cosolvent	-	9.06±1.44	11.41±1.13	11.53±0.96	10.22±1.27	13.15±1.30	12.04±1.61
Aspirin	200	9.89±1.17	10.54±0.73	9.09±1.26	8.79±0.75	11.03±1.04	12.05±1.42
Morphine sulphate	10	12.07±0.91	23.62±1.18**	21.60±0.90**	28.27±2.29**	15.03±1.90*	12.19±1.13
C. aeruginosa	100	9.03±1.12	11.01±1.23	10.65±0.98	8.48±1.52	11.28±1.23	11.20±1.38
(methanol	200	9.52±1.23	12.00±1.59	9.85±1.47	10.69±1.10	12.94±1.54	13.62±1.77
extract)	400	8.96±0.65	11.11±1.24	10.26±1.16	9.34±0.73	9.16±0.62	10.97±1.19
Cosolvent	-	9.84±0.81	13.84±1.81	10.13±1.17	12.55±0.94	13.49±1.62	11.94±1.60
Aspirin	200	10.21±0.80	12.59±0.84	10.86±1.55	9.74±0.96	11.39±1.86	10.21±1.53
C. aeruginosa	100	9.42±0.79	11.15±1.49	13.74±1.33	13.49±1.14	13.00±1.54	13.56±2.31
(water	200	9.13±0.78	11.21±0.76	14.13±1.91	15.13±2.56	11.23±1.05	12.48±1.12
extract)	400	9.75±0.48	13.69±1.11	12.22±1.18	11.61±0.98	11.91±0.66	12.52±1.43

Beginning thirty min after oral administration of test agents (or 15 min after morphine injection, s.c.) the nociceptive response was measured every 15 min over a 60-min period. T_b = Time before oral adminstration of test agent. Each datum represents the mean latency of nociceptive response (sec) ± S.E.M. (n=10). * p<0.05, **p<0.01 compared with the control group (Dunnett's test).

Effects of *C. aeruginosa* on nociceptive responses

Writhing test

Oral administration of the chloroform extract and methanol extract of *C. aeruginosa* (100, 200 and 400 mg/kg) dose-dependently attenuated the number of writhings induced by an intraperitoneal injection of 0.6% acetic acid (Table 1). Aspirin (200 mg/kg, p.o.), standard analgesic drug, also reduced the number of writhings in mice. But the water extract of *C. aeruginosa* had no effect on acetic acid-induced writhing response in mice.

Hot plate test

The mean latency of nociceptive responses to thermal stimuli is summarized in Table 2. Neither the chloroform extract, methanol extract, water extract of *C. aeruginosa* (100, 200 and 400 mg/kg, p.o.) nor aspirin (200 mg/kg, p.o.) significantly exerted protective effects on heat-induced pain in mice. In contrast, a centrally acting analgesic drug, morphine sulfate (10 mg/kg, s.c.) markedly increased the pain latency.

Formalin test

The chloroform extract of *C. aeruginosa* dose-dependently decreased the licking activity in the late phase but not in the early phase of formalin-induced pain (Table 3). Aspirin (200 mg/kg) also suppressed only in the late phase. The methanol extract and the water extract of *C. aeruginosa* had no effect on either the early or late phases. In contrast, the reference drug morphine sulfate (10 mg/kg) significantly reduced the licking activity in both phases of formalin-induced nociception.

Effect of *C. aeruginosa* on yeast-induced fever in rats

None of the extracts of *C. aeruginosa* had any significant effects on pyrexia induced by yeast in rats. Aspirin (200 mg/kg) significantly decreased the fever induced by yeast in rats (Table 4).

Effect of *C. aeruginosa* on carrageenin-induced paw edema in rats

No significant suppression on carrageenin-induced paw edema was observed after oral administration of all extracts (100, 200, 400 and 800 mg/kg, p.o.) from *C. aeruginosa* even at the

Table 3. Effect of the chloroform extract, methanol extract and water extract from *C. aeruginosa*, aspirin and morphine on licking activity in mice.

Licking time (sec)											
Cosolvent		Aspirin		Morphine		*C. aeruginosa* extract	Dose (mg/kg p.o.)				
							Early phase			Late phase	
Early phase	Late phase	Early phase	Late phase	Early phase	Late phase		100	200	400	100	200	400
50.97±	59.33±	27.62±	9.89±	-	-	Chloroform ext.	45.76±	36.46±	45.06±	53.66±	17.50±	7.41±
4.35	8.02	9.14	16.83*				3.33	3.82	5.62	13.06	5.84*	4.24*
77.64±	35.71±	65.19±	7.36±	2.8±	0.0±	Methanol ext.	77.69±	58.50±	66.34±	60.43±	47.01±	40.67±
6.51	4.29	2.74	4.01*	0.35**	0.0**		4.53	3.58	8.14	7.14	9.10	12.72
67.67±	78.90±	62.32±	28.64±	-	-	Water ext.	62.14±	73.11±	69.72±	52.11±	60.48±	49.79±
4.05	9.68	12.90	25.82*				4.47	3.85	3.74	12.90	9.89	6.19

Thirty min after drug administration (p.o.) (or 15 min after morphine injection, s.c.), 2.5 % formalin was subcutaneously injected to a hindpaw in a volume of 20 µl. Each datum represents the mean of licking time ± S.E.M. from 10 mice in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection. * p<0.05, **p<0.01 compared with the control group (Dunnett's test).

Table 4. Effect of the chloroform extract, methanol extract and water extract from *C. aeruginosa* and aspirin on brewer's yeast-induced fever in rats.

Drug	Dose (mg/kg, p.o.)	Average rectal temperature (ºC)					
		0	1hr	2hr	3hr	4hr	5hr
Cosolvent	-	38.0±0.2	37.3±0.2	37.0±0.2	36.7±0.3	36.7±0.1	36.7±0.1
Aspirin	200	37.9±0.1	36.9±0.2	36.4 ±0.2	36.0±0.2*	36.0±0.2*	35.9±0.2*
C. aeruginosa	100	38.0±0.2	37.0±0.1	36.9 ±0.2	36.7±0.2	36.7±0.2	36.5±0.2
(chloroform extract)	200	38.0±0.2	37.3±0.2	37.0 ±0.2	37.0±0.2	36.8±0.2	36.7±0.2
	400	37.8±0.1	37.1±0.1	37.0 ±0.1	36.8±0.1	36.8±0.1	36.8±0.1
Cosolvent	-	37.6±0.1	37.1±0.1	36.8±0.1	36.6±0.2	36.6±0.1	36.7±0.1
Aspirin	200	37.8±0.1	36.6±0.1*	36.3 ±0.1*	36.1±0.1*	36.0±0.1*	35.9±0.1*
C. aeruginosa	100	37.7±0.2	37.2±0.1	37.0 ±0.1	36.8±0.1	36.7±0.1	36.7±0.1
(methanol extract)	200	37.4±0.1	37.2±0.1	36.8 ±0.1	36.8±0.1	36.8±0.2	36.8±0.2
	400	37.4±0.2	36.9±0.2	36.7 ±0.1	36.6±0.1	36.5±0.2	36.7±0.2
Cosolvent	-	38.3±0.2	37.6±0.2	37.0±0.2	36.8±0.2	36.8±0.1	36.9±0.1
Aspirin	200	38.2±0.1	37.3±0.2	36.7 ±0.2	36.5±0.3*	36.4±0.3*	36.4±0.3*
C. aeruginosa	100	38.3±0.1	37.6±0.1	37.3 ±0.1	37.0±0.2	37.0±0.1	37.1±0.1
(water extract)	200	38.3±0.2	37.5±0.2	37.1 ±0.2	37.1±0.1	37.0±0.1	37.0±0.1
	400	38.3±0.2	37.6±0.2	37.5 ±0.2	37.1±0.2	37.2±0.2	37.2±0.1

Twenty percent yeast suspension was subcutaneously injected into the dorsum region of rats. Seventeen hours after injection, rectal temperature was measured (time 0) and then drugs were orally administered. The temperature was again measured at 1, 2, 3, 4 and 5 hr after drug administration. Each datum represents the mean rectal temperature (ºC) ± S.E.M. (n = 6). *p<0.05, compared with the control group (Dunnett's test).

high dose (800 mg/kg), while aspirin (200 mg/kg) significantly reduced the carrageenin-induced paw edema in rats (Table 5).

Discussion

The results demonstrate that the chloroform and the methanol extracts obtained from the rhizomes of *C. aeruginosa* attenuated nociceptive responses to chemical stimuli (acetic acid-induced writhing response in mice). Only the chloroform extract of *C. aeruginosa* suppressed the licking activity of the late phase in the formalin test in mice. None of the extracts had any significant effects on heat-induced pain in mice, yeast-induced fever or paw edema induced by carrageenin in rats.

The chloroform extract and the methanol extract of *C. aeruginosa* exerted protective action against nociception in the writhing test similarly to the reference peripheral analgesic compound, aspirin. This test is generally used for screening of antinociceptive effect (Koster *et al.*, 1959; Hendershot & Forsaith, 1959). These results suggest that the chloroform and methanol extracts apparently possess antinociceptive activities.

Thermic painful stimuli are known to be selective to centrally, but not peripherally, acting analgesic drugs (Chau, 1989). In the present study, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in this test, while all extracts of *C. aeruginosa* failed to affect the response. These findings, therefore, suggest that the apparent antinociceptive action of the active compound (s) in the chloroform extract and the methanol extract of *C. aeruginosa* is mediated through peripheral but not central mechanism(s).

The formalin test is another pain model, which assesses the way an animal responds to moderate, continuous pain generated by injured tissue (Tjolsen, 1992). The effects of drugs on the licking responses in the early and late phases

Table 5. Effect of the chloroform extract, methanol extract and water extract from *C. aeruginosa* and aspirin on carrageenin-induced paw edema in rats.

Drug	Dose (mg/kg, p.o.)	Paw volume (ml)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Cosolvent	-	3.48±0.29	4.67±0.10	5.24±0.11	6.13±0.20	6.38±0.17	6.72±0.10
Aspirin	200	3.58±0.34	4.83±0.22	4.88±0.13	5.14±0.21*	5.54±0.19*	5.95±0.12*
C. aeruginosa	100	3.87±0.17	4.74±0.13	5.38±0.28	5.92±0.16	6.45±0.14	6.46±0.17
(chloroform extract)	200	4.03±0.23	5.05±0.19	5.31±0.15	5.80±0.22	6.13±0.14	6.33±0.15
	400	4.59±0.16	4.87±0.11	5.32±0.12	6.18±0.26	6.51±0.18	6.67±0.19
	800	4.31±0.13	4.87±0.13	5.49±0.29	6.01±0.24	6.42±0.24	6.59±0.28
Cosolvent	-	3.80±0.10	5.04±0.18	5.89±0.19	6.48±0.18	6.94±0.22	7.46±0.28
Aspirin	200	3.97±0.23	4.63±0.29	4.74±0.26*	5.17±0.17*	5.59±0.13*	6.02±0.20*
C. aeruginosa	100	4.32±0.12	5.13±0.18	5.81±0.37	6.36±0.29	6.87±0.26	7.20±0.21
(methanol extract)	200	3.78±0.20	4.77±0.14	5.78±0.20	6.68±0.19	7.18±0.32	6.86±0.16
	400	4.13±0.12	4.91±0.11	5.47±0.21	6.39±0.19	6.86±0.26	7.14±0.24
	800	3.96±0.28	4.96±0.09	5.92±0.15	6.62±0.18	7.06±0.22	7.09±0.19
Cosolvent	-	3.67±0.08	4.63±0.09	5.95±0.34	6.83±0.20	7.57±0.27	7.36±0.33
Aspirin	200	3.73±0.14	4.45±0.15	4.67±0.14*	4.56±0.16*	5.02±0.25*	5.03±0.25*
C. aeruginosa	100	3.85±0.14	4.61±0.09	5.84±0.33	6.36±0.40	6.69±0.47	6.40±0.38
(water extract)	200	4.04±0.08	4.75±0.06	5.92±0.13	6.58±0.17	7.17±0.29	7.00±0.26
	400	4.02±0.10	4.77±0.20	5.97±0.46	6.58±0.41	6.94±0.36	6.40±0.19
	800	3.95±0.14	4.75±0.11	5.78±0.31	6.37±0.33	6.93±0.49	6.52±0.57

The initial hind paw volume of the rat was determined volumetrically. Thirty min after test agent administration (p.o.), 1% carrageenin in saline was subcutaneously injected in a volume of 0.1 ml into the right hind paw at time 0 and the paw volume was measured at 1 hr intervals for 5 hr. Each point represents the mean ± S.E.M of 6 rats.
***p<0.05, compared with the control group (Dunnett's test).**

reportedly represent antinociceptive action on sensory receptor stimulation and anti-inflammatory action, respectively (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987). The chloroform extract of *C. aeruginosa* produced a dose-related reduction of licking activity in the late phase of formalin-induced pain whereas the methanol extract of *C. aeruginosa* had the effect only on the writhing test but not on the formalin model. It is possible that most of the active compound (s) may be included in the chloroform extract. The essential oil of the rhizomes and leaves of *C. aeruginosa* have been identified to be composed of 1,8-cineole, curzerenone, furanogermenone, camphor, (Z)-3-hexenol, zedoarol, furanodienone, curcumenol, isocurcumenol, beta-elemene, curzerene and germacrone (Xuan Dung *et al*., 1995; Sirat *et al*., 1998b; Bin Jantan *et al*., 1999; Jirovetz *et al*., 2000). The 1,8-cineol compound, a terpenoid oxide present in many plant essential oils displayed antinociceptive and anti-inflammatory effects in experimental animals (Santos & Rao, 2000). In addition, germacrone and curzerenone also exerted anti-inflammatory activity in carrageenin-induced hind paw edema in rats (Claeson *et al*., 1993). As all of these compounds are soluble in chloroform, some of these compounds may be involved in the activity of the chloroform extract of *C. aeruginosa*. Although it did not show inhibitory effect on paw edema induced by carrageenin in rats, it is possible that some active volatile components may be lost during the process of extraction of the chloroform extract by thermic conventional method.

Since the chloroform extract of *C. aerugi-*

nosa suppressed writhing and licking activity of the late phase in mice but had no significant effects on yeast-induced pyrexia or paw edema induced by carrageenin in rats, it also had no inhibitory activity on COX assay *in vitro* (unpublished observation), as compared to the reference drug aspirin, a nonsteroidal anti-inflammatory drug possessing analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclooxygenase pathway (Vane, 1987). As nociceptors are exposed to noxious stimuli, some chemical pain mediators such as bradykinins, prostaglandins, histamine, serotonin and substance P are released from damaged tissues (Gebhart & McCormack, 1994). Thus, the antinociceptive activity of the active compound (s) included in the chloroform extract of *C. aeruginosa* rhizome may act on or involve some of those pain mediators-that is, a different mechanism from that of aspirin.

Based on these results, we conclude that the chloroform extract of *C. aeruginosa* rhizome possesses analgesic effect, and that its action on nociception may be different from that of aspirin.

Acknowledgments

The authors are very grateful to Prince of Songkla University for financial support.

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