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New medicinal properties of mangostins: Analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana* L.

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ABSTRACT

The fruit hull of *Garcinia mangostana* L. contains oxygenated and prenylated phenol derivatives, such as xanthones or xanthen-9H-ones, and is used by people in Southeast Asia as a traditional medicine for the treatment of abdominal pain, dysentery, wound infections, suppuration, and chronic ulcer. We isolated the active ingredients from the crude ethanol extract of *G. mangostana* L. (CEM) and investigated their analgesic effects and underlying mechanisms. CEM at intragastric (i.g.) doses of 0.5, 1, and 3 g/kg clearly exhibited antinociceptive effects in the hot-plate and acetic acid-induced writhing tests in mice. Two isolated compounds, α-mangostin and γ-mangostin, exhibited analgesic effects at doses of 25 and 50 mg/kg (i.g.) in the hot-plate and formalin tests, respectively. CEM at doses of 0.5, 1, and 3 g/kg significantly inhibited xylene-induced release of inflammatory mediators. CEM, α-mangostin, and γ-mangostin each dose-dependently demonstrated the ability to scavenge reactive oxygen species. In conclusion, our results demonstrate that CEM and mangostins possess potent peripheral and central antinociceptive effects in mice and suggest that xanthones may be developed as novel analgesics and anti-inflammatory drugs.

1. Introduction

Herbaceous plants are important sources for many biologically active compounds that have strong therapeutic effects. Mangosteen (*Garcinia mangostana* L. [GML], "the queen of fruits") is a tropical evergreen tree that belongs to the family Guttiferae and is widely distributed throughout India, Myanmar, Malaysia, Philippines, Sri Lanka, and Thailand (Pedraza-Chaverrí et al., 2008). People in these countries have used the pericarp (peel, rind, and hull) or the ripe fruit of mangosteen as a traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, wound infection, suppuration, and chronic ulcer (Suksamram et al., 2006). *Garcinia* species are known to be rich in secondary metabolites, such as prenylated and oxygenated xanthones. Xanthones or xanthen-9H-ones are secondary metabolites found in the pericarp, whole fruit, bark, and leaves of GML and some other higher plant families, fungi, and lichens (Pedraza-Chaverrí et al., 2008; Peres et al., 2000). Previous studies have shown that α-mangostin, β-mangostin, γ-mangostin, garcinone E, 8-deoxygartanin, and gartanin are the most abundant xanthones. Antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral effects have been demonstrated for xanthones isolated from GML (Pedraza-Chaverrí et al., 2009; Chomnawang et al., 2007; Chen et al., 2008).

α-Mangostin, which was firstly isolated in 1855, has been confirmed to be a competitive antagonist of the histamine H_1 receptor (Chairungsrilerd et al., 1996) and possesses many biological effects, such as antibacterial and anti-inflammatory activity (Tewtrakul et al., 2009), inhibition of oxidative damage (Mahabusarakam et al., 2000), and antimicrobial and weak antioxidant activity (Iikubo et al., 2002; Chen et al., 2008). γ-Mangostin, which was first isolated by Jefferson et al. in 1970 (Jefferson et al., 1970) from the pericarp of *G. mangostana* L., exhibits serotonin-2A (5-hydroxytryptamine-2A, 5-HT_{2A}) receptor antagonist effects, inhibits lipopolysaccharide-stimulated nitric oxide (NO) production, has anti-inflammatory effects, and inhibits cyclooxygenase (COX) and prostaglandin E2 (PGE2) synthesis in C_6 rat glioma cells (Chairungsrilerd et al., 1996; Chairungsrilerd et al., 1998; Chen et al., 2008; Nakatani et al., 2002). Considering these confirmed effects of mangostins and the traditional treatment with GML, we hypothesized that the crude ethanol extract of *G. mangostana* L. (CEM), α-mangostin, and γ-mangostin would each produce analgesic effects on central and peripheral pain, and we further explored the underlying mechanisms of action of CEM, α-mangostin, and γ-mangostin.

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2. Methods

2.1. Reagents and materials

All chemical reagents were of analytical grade unless otherwise specified. Methanol, reagent alcohol, acetic acid, $FeSO_4 \cdot 7H_2O$, dimethyl sulfoxide (DMSO), ethyl acetate (EtOAC), petroleum ether, xylene, pyrogallol, 30% H_2O_2, and sodium salicylate were purchased from Guangzhou Chemical Reagent Factory (Guangdong, China). Indomethacin, a nonselective COX inhibitor, and tramadol hydrochloride were purchased from Guangdong South of China Pharmacological Corp. (Guangdong, China).

2.2. Preparation of plant extracts and identification

The fruit hull of mangosteen (GML) was purchased from a local market and was cleaned with distilled water and dried in a vacuum drying cabinet (DZG-6050, Shanghai Shenxin Experimental Apparatus Corp., Shanghai, China). After milling, 4.37 kg of dried powder was immersed in 6.4 L of 95% ethanol at room temperature. The extraction was repeated three times within 1 week, and the combined solution was concentrated. CEM was then obtained. One part of CEM was extracted in triplicate with an optimum water:EtOAC (1:1) ratio. Fraction III was acquired with a silica gel column eluted with an EtOAC-petroleum ether ratio of 25:75 on an ethyl acetate extract phase. Methanol was added to fraction III, which was then recrystallized. Compound 1 (α-mangostin) was then acquired. Another part of the ethyl acetate extract phase was chromatographed through a silica gel column eluted twice with a EtOAC–petroleum ether ratio of 35:65 and eluted twice with a methanol–chloroform ratio of 3:97. Compound 2 (γ-mangostin) was then acquired. The residual part of CEM was used for pharmacological experiments.

The structures of the isolated compounds were determined with ^{13}C-nuclear magnetic resonance (NMR), ^{1}H NMR, DEPT NMR, and D_2O exchange NMR (Instrumental Analysis and Research Center of Sun Yat-sen University) and compared with those of known compounds (Mahabusarakam et al., 1987). The purity of the compounds was confirmed by high-performance liquid chromatography (HPLC) with an optimal concentration of 1 mg compound: 1 mL methanol using a Betasil C18 column.

2.3. Animals

Female KM mice, weighing 18–27 g, were purchased from the Experimental Animal Center of Sun Yat-sun University. Mice were housed in plastic cages with free access to food and water at room temperature (25–27 °C) and constant humidity (55 ± 5%). The experimental procedures were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication No. 85-23, revised 1985).

2.4. Hot-plate test

The hot-plate test was performed as previously described (Ridtitid et al., 2008) to measure hindlimb licking or flicking latencies following exposure to the hot-plate. Only mice that showed a nociceptive response within 30 s were used in the experiment. The percentage of mice that failed to show a tail-flick latency ≤ 30 s was 12.3%. Measurements were taken 1, 2, 3, and 4 h after drug administration. Mice were placed on the hot-plate, which was maintained at 55 ± 0.5 °C. The latency of the nociceptive response of each mouse, identified as the time to lick or flick a hind limb, was recorded. The cut-off time of observation was 60 s. Inhibition of hot-plate paw-licking responses was expressed as the percentage of the maximal possible effect (% MPE), calculated as $([T1 - T0]/[T2 - T0]) \times 100$, with $T0$ and $T1$ representing the hot-plate paw-licking latencies before and after the administration of test drugs or vehicle, respectively. The cut-off time ($T2$) was set at 60 s for the hot-plate test. Hot-plate tests were performed twice. In experiment 1, the test groups were administered intragastrically (i.g.) with three doses of CEM (3.0, 1.0, and 0.5 g/kg), and mice in the control group received saline solution (10 mL/kg, i.g.), with indomethacin (10 mg/kg, i.g.) as the reference drug. In experiment 2, the test groups were administered α-mangostin (25 and 50 mg/kg, i.g.) or γ-mangostin (25 and 50 mg/kg, i.g.), and mice in the control group received the cosolvent (10 mL/kg, i.g.; propylene glycol:DMSO:distilled water, 1:1:9), with indomethacin (10 mg/kg) and tramadol hydrochloride (50 mg/kg, i.g.) as the reference drugs, respectively.

2.5. Acetic acid-induced writhing test

This test was carried out to evaluate analgesic effects using the modified method described by Koster et al. (1959). The test groups were administered three doses of CEM (3.0, 1.0, and 0.5 g/kg, i.g.). Sixty minutes later, the animals were treated with 0.8% acetic acid in 0.9% normal saline (10 mL/kg) via intraperitoneal injection to induce the characteristic writhing response. Indomethacin (10 mg/kg, i.g.) or saline solution (10 mL/kg, i.g.) was administered to mice in the reference or control group, respectively. The mice were observed, and the number of abdominal constrictions and stretching episodes were counted for a 20 min period. The responses in the drug-treated groups were compared with the control group. The percentage of inhibition of the number of writhing episodes was calculated as the inhibition rate ($\% = [S - s]/S \times 100$), with S representing the average twisting episodes of the control group and s representing the average twisting episodes of the test or reference groups.

2.6. Formalin test

Animals were pretreated intragastrically with different doses of α-mangostin (25 and 50 mg/kg) or γ-mangostin (25 and 50 mg/kg) prior to performing the formalin test (Hunskaar et al., 1985). The control group received cosolvent (10 mL/kg, i.g.; propylene glycol: DMSO:distilled water, 1:1:9), and the reference groups were treated with tramadol hydrochloride (50 mg/kg, i.g.) or indomethacin (10 mg/kg, i.g.), respectively. Sixty minutes after treatment, 20 μL of a 5% formalin solution was injected subcutaneously under the plantar surface of a hind paw in each mouse. Following formalin injection, the animals were immediately placed in an observation beaker. The time spent licking/flinching and biting the injected paw was measured with a stopwatch and was considered a sign of nociception. The first phase of the nociceptive response normally peaked from 0 to 5 min, and the second phase normally peaked from 20 to 45 min after formalin injection (total of 20–25, 30–35, and 40–45 min recorded), reflecting the direct effect on nociceptors and inflammatory nociceptive responses, respectively (Hunskaar and Hole, 1987). The inhibition rate of the licking time was calculated as $\% = (t - T)/t \times 100$, with t representing the licking time of the control group and T representing the licking time of the test or reference group.

2.7. Xylene-induced ear edema test

The anti-inflammatory effects of CEM were assessed based on the method introduced by Winter et al. (1962). CEM (3.0, 1.0, and 0.5 g/kg, i.g.) was administered to mice in each of the groups 60 min prior to the test. Indomethacin (10 mg/kg, i.g.) was used in the reference group, and the same volume of saline administered intragastrically was used in the control group. Xylene, 0.03 mL, was then smeared on the right ear of each mouse to induce edema. The left ear remained untreated as the null treatment. Ear edema was measured 4 h after xylene administration using an electronic balance (BS 200S-WEI, Sartorius). The degree of edema was evaluated

by the delta weight ($a-b$), with a and b representing the weight of the part of the right and left ears subjected to a 7 mm diameter hole punch to obtain symmetrical tissue after the mouse was sacrificed by decapitation.

2.8. Antioxidant experiment

2.8.1. Hydroxyl radical-scavenging (·OH) activity

The scavenging activity of α-mangostin, γ-mangostin, and CEM against hydroxyl radicals (·OH) was conducted in the $FeSO_4+H_2O_2$ system introduced by Gomez-Vargas et al. (1998). Briefly, ·OH was derived from the chemical reaction between $FeSO_4+H_2O_2$ (3 mL reaction solution containing 0.15 mol/L $FeSO_4$, 6 mmol/L H_2O_2, and 2 mmol/L sodium salicylate) and different concentrations of CEM, α-mangostin, or γ-mangostin. The product (·OH) from oxidizing the salicylic acid was directly measured by absorption at an optical density of 510 nm, which represents the amount of ·OH. ·OH was generated by incubating the mixture at 37 °C for 60 min after adding H_2O_2 to the reaction mixture. The scavenging activity of ·OH was expressed as $\% = (A1 - As)/(A1) \times 100$, with As representing the absorbance of the reaction mixture with the sample and $A1$ representing the absorbance of the reaction mixture containing sodium salicylate, $FeSO_4$, and H_2O_2.

2.8.2. Superoxide radical-scavenging ($\cdot O_2^-$) activity

The superoxide anion $\cdot O_2^-$ was generated from the auto-oxidation of pyrogallol in a basic solution as described by Marklund and Marklund (1974) with some modifications. The superoxide radical-scavenging activity of mangostins was estimated using spectrophotometric monitoring of the inhibition of pyrogallol autoxidation. 10 μL of 45 mmol/L pyrogallol solutions was added to a tube containing 4.5 mL of different doses of α-mangostin and γ-mangostin. Absorbance was measured at 325 nm every 20 s using a spectrophotometer within 4 min. Antioxidant activity was determined as the rate of pyrogallol autoxidation, which was calculated as absorbance in the presence or absence of pyrogallol and mangostin samples. The scavenging activity of superoxide radicals was expressed as $\% = (\Delta A1/\Delta t - \Delta A2/\Delta t)/(\Delta A1/\Delta t) \times 100$, with $\Delta A1/\Delta t$ representing the pyrogallol autoxidation rate without sample and $\Delta A2/\Delta t$ representing the pyrogallol autoxidation rate with sample.

2.9. Data analysis

Statistical analysis was performed using Origin 7.5 software (Origin Lab Corp, Northampton, MA, USA). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test. Values of $p<0.05$ were considered statistically significant. Data are expressed as mean ± standard deviation (SD) unless otherwise specified.

3. Results

3.1. Identification of main active ingredients of CEM

Based on the data acquired from NMR and mass spectroscopy, compound 1 consisted of five $-CH_3$, two $-CH_2$, four –CH, twenty quaternary carbons, and three –OH, with a molecular weight of 410. Compound 2 consisted of four $-CH_3$, two $-CH_2$, four –CH, twelve quaternary carbons, and three –OH, with a molecular weight of 396. According to a previous report (Mahabusarakam et al., 1987), we identified compound 1 and compound 2 as α-mangostin and γ-mangostin, respectively (Fig. 1). The purity of α-mangostin and γ-mangostin was determined by HPLC to be 91.18% and 92.07%, respectively. And the extraction yield of α-mangostin and γ-mangostin from CEM was 0.69% and 0.14%, respectively.

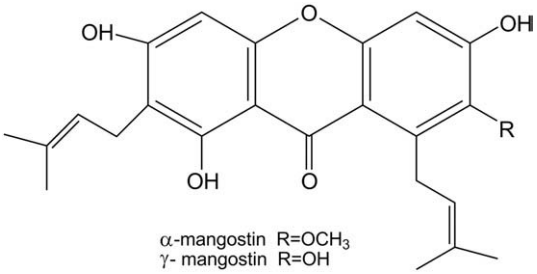

Fig. 1. Structures of α-mangostin and γ-mangostin isolated from the fruit hull of *Garcinia mangostana* L.

Additionally, our modified technique of extraction and isolation was based on a previous report (Mahabusarakam et al., 1987). We used 95% ethanol rather than benzene as the extraction solvent to reduce the toxicity of residual benzene. The 95% ethanol extraction solvent had nearly the same yield as previous reports.

3.2. Analgesic effect of CEM in mice

3.2.1. Hot-plate test

As shown in Fig. 2A, oral administration of CEM at a dose of 0.5 g/kg produced significant inhibition of the hot-plate response at the 2nd and 4th h, which reached a peak 4 h after administration (48.1 ± 16.28% MPE, $n=10$; one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$). Both 1.0 and 3.0 g/kg CEM markedly prolonged latencies of the hot-plate paw-licking response from the 1st to 4th h after oral administration, reaching a peak of 57.0 ± 17.65% MPE at the 4th h in the 1.0 g/kg CEM-treated group ($n=10$) and 76.5 ± 12.68% MPE at the 2nd h in the 3.0 g/kg CEM-treated group ($n=10$) (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$). Overall, CEM dose-dependently increased latencies of the hot-plate paw-licking response. The antinociceptive effect of CEM was fast and long-lasting, demonstrated by the fact that CEM produced marked antinociception as early as the 1st h after oral administration, and the effects remained significant up to the 4th h after administration. Although the latency of the hot-plate paw-licking response in the indomethacin-treated group (10 mg/kg) was greater than in the control group, it did not reach statistical significance (one-way ANOVA followed by Tukey *post hoc* test, $p>0.05$, $n=10$).

3.2.2. Acetic acid-induced writhing test

In the acetic acid-induced writhing test, CEM and indomethacin inhibited the writhing response and decreased writhing times at all doses tested. Writhing times in the CEM- (0.5 g/kg, $n=10$) and indomethacin- (10 mg/kg, $n=10$) treated groups showed significant differences compared with the saline control group (28.4 ± 4.14 s, $n=10$; one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$). The differences produced by 1.0 g/kg ($n=10$) and 3.0 g/kg ($n=10$) CEM were much more pronounced compared with the saline control group (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$). Fig. 2B shows that CEM produced concentration-dependent pain inhibition. Writhing times in the 0.5, 1.0, and 3.0 g/kg CEM-treated groups were 15.1 ± 3.23 s (47%), 7.22 ± 2.33 s (76%), and 0.89 ± 0.68 s (97%), respectively, whereas writhing time in the indomethacin (10 mg/kg) group was 14.2 ± 2.48 s (50%).

3.3. Analgesic effect of mangostins in mice

3.3.1. Hot-plate test

As shown in Fig. 3A, maximum antinociception of 38.0 ± 19.45% MPE was observed at the 1st h after administration of 50 mg/kg α-mangostin (α1; one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$, $n=10$). 25 mg/kg α-mangostin (α2) was not

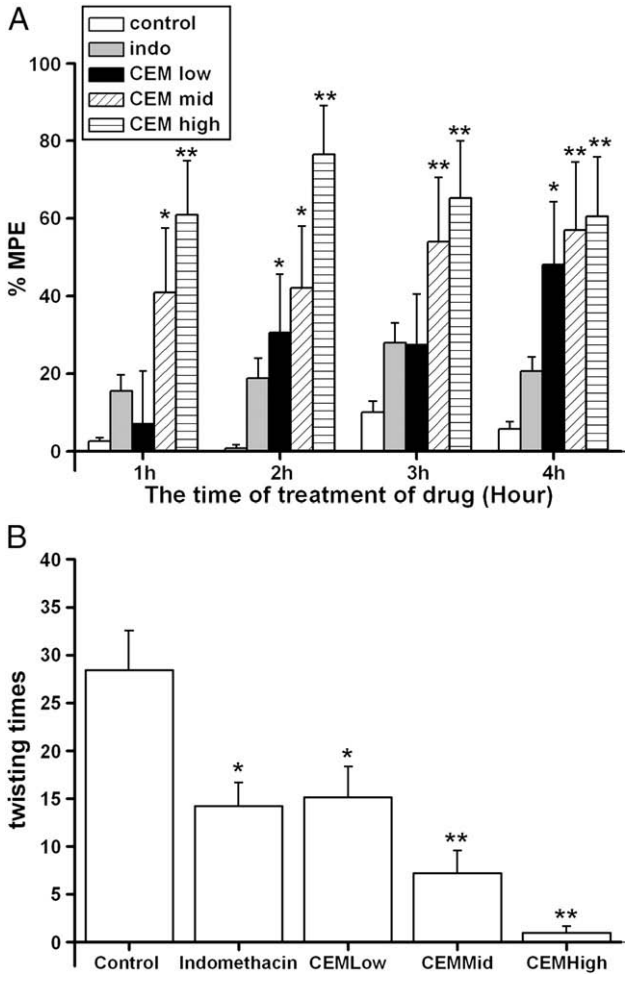

Fig. 2. Effects of CEM at different concentrations on hot-plate-induced paw withdraw and acetic acid-induced writhing in mice ($n=10$/group). Error bars indicate standard deviation. $^{*}p<0.05$, $^{**}p<0.01$, compared with saline control group (one-way ANOVA followed by Tukey *post hoc* test. (A) Effects of CEM at different doses (CEM low, 0.5 g/kg; CEM mid, 1.0 g/kg; CEM high, 3.0 g/kg) on the time-course of paw withdraw in the hot-plate test. The control group received saline solution (10 mL/kg), and the reference drug was indomethacin (10 mg/kg). (B) Effects of CEM at different doses (CEM low, 0.5 g/kg; CEM mid, 1.0 g/kg; CEM high, 3.0 g/kg) on writhing times induced by acetic acid. The control group received saline solution (10 mL/kg), and the reference drug was indomethacin (10 mg/kg).

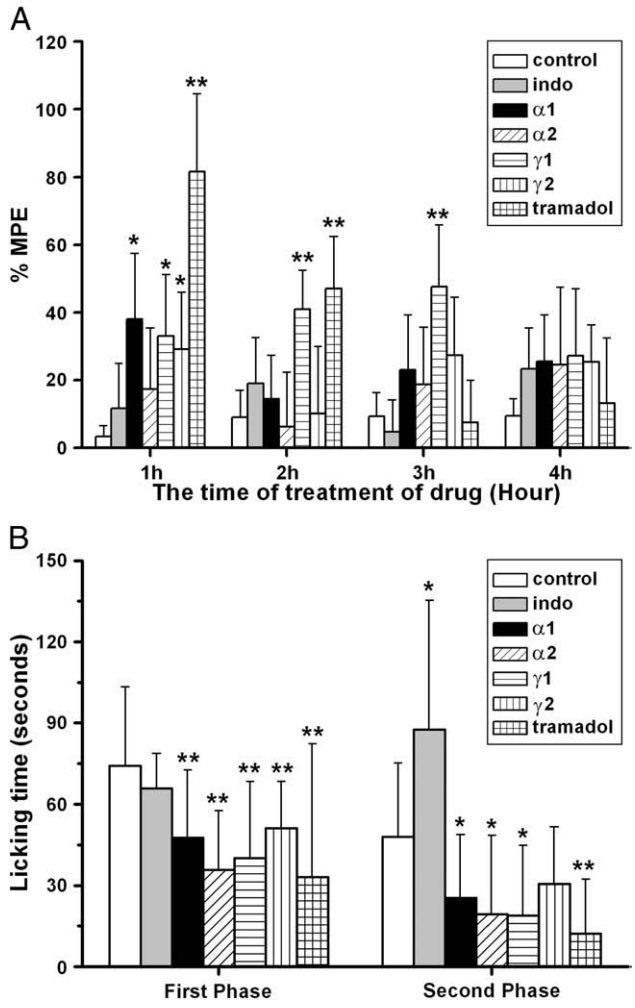

Fig. 3. Effects of α- and γ-mangostin at different concentrations on hot-plate-induced paw withdraw and formalin-induced licking in mice ($n=10$/group). Error bars indicate standard deviation. $^{*}p<0.05$, $^{**}p<0.01$, compared with saline control group (one-way ANOVA followed by Tukey *post hoc* test). (A) Effects of α-mangostin (α1, 50 mg/kg, α2, 25 mg/kg) and γ-mangostin (γ1, 50 mg/kg; γ2, 25 mg/kg) on the time-course of paw withdraw in the hot-plate test. The control group received the cosolvent (10 mL/kg, propylene glycol:DMSO:distilled water, 1:1:9), and the reference drugs were indomethacin (10 mg/kg) and tramadol hydrochloride (50 mg/kg), respectively. (B) Effects of α-mangostin (α1, 50 mg/kg; α2, 25 mg/kg) and γ-mangostin (γ1, 50 mg/kg; γ2, 25 mg/kg) on licking time induced by formalin in mice. The control received the cosolvent (10 mL/kg, propylene glycol:DMSO:distilled water, 1:1:9), and the reference drugs were indomethacin (10 mg/kg) and tramadol hydrochloride (50 mg/kg), respectively.

effective at inducing antinociception in the hot-plate test at all test times after administration (one-way ANOVA followed by Tukey *post hoc* test, $p>0.05$, $n=10$). γ-Mangostin significantly increased the latency in the hot-plate test at a dose of 50 mg/kg (γ1, $n=10$), an effect that lasted for 3 h after administration. Maximum antinociception of 47.6 ± 18.28% MPE was observed at the 3rd h after administration (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$). 25 mg/kg γ-mangostin (γ2, $n=10$) also significantly increased latency in the hot-plate test 1 h after administration, which reached a peak of 29.2 ± 16.74% MPE (one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$). Indomethacin (10 mg/kg, $n=10$) did not increase latency in the hot-plate test. Tramadol hydrochloride produced a marked antinociceptive effect in the hot-plate test at 50 mg/kg (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$, $n=10$), presumably through activation of μ opioid receptors and inhibition of serotonin reuptake (Oliva et al., 2002).

3.3.2. Formalin test

The formalin test consisted of two different phases. The first phase acts in the periphery through activation of nociceptive neurons by a direct effect of formalin, and the second phase occurs through activation of central horn neurons at the spinal cord level (Ridtitid et al., 2008). Fig. 3B shows that mangostins produced antinociception in both the first phase (neurogenic) and second phase (inflammatory) of the formalin test. α-Mangostin significantly inhibited both the first and second phases of formalin-induced licking, with inhibition of 35.8% and 47.1% at a dose of 50 mg/kg (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$ and $p<0.05$, respectively, $n=10$) and 51.7% and 59.6% at a dose of 25 mg/kg (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$ and $p<0.05$, respectively, $n=10$) for the first and second phases, respectively. The effect of 25 mg/kg of α-mangostin was more potent than 50 mg/kg in the formalin test. γ-Mangostin at a dose of 50 mg/kg was also effective at producing antinociception both in the first and second phases of the formalin test, with inhibitions of 45.7% and 60.6% in the first and second phases (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$ and $p<0.05$, $n=10$), respectively. However, the antinociceptive effects

of γ-mangostin at a dose of 25 mg/kg were significant in the first phase (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$, $n=10$;% inhibition = 31.0%) and nonsignificant in the second phase (one-way ANOVA followed by Tukey *post hoc* test, $p>0.05$, $n=10$) of the formalin test. Tramadol hydrochloride at a dose of 50 mg/kg significantly inhibited nociception in both phases of the formalin test (one-way ANOVA followed by Tukey *post hoc* test, $p<0.01$, $n=10$; see also Martindale et al., 2001). These results are inconsistent with a previous report showing that (F)-tramadol and its enantiomers significantly reduced the duration of nociceptive behaviors in the second phase of the formalin test (Oliva et al., 2002). Indomethacin (10 mg/kg) modestly but nonsignificantly reduced licking time in the first phase (one-way ANOVA followed by Tukey *post hoc* test, $p>0.05$, $n=10$) but significantly increased licking time (one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$; Fig. 3B) and produced analgesia in the second phase of the formalin test. Cyclooxygenase and lipoxygenase (LOX) share a common arachidonic acid substrate, and inhibition of COX enzymes by indomethacin has been suggested to lead to a shunt of arachidonic acid metabolism toward the 5-LOX pathway, resulting in increased production of leukotrienes which are the second main family of arachidonic acid derivatives. This cascade then leads to changes in vascular permeability occurring during acute inflammation and plays a role in nonsteroidal anti-inflammatory drug (NSAID)-induced gastrointestinal damage (Martel-Pelletier et al., 2003).

3.4. Assessment of some mechanisms involved in the antinociceptive effect of CEM and mangostins

3.4.1. Effects of CEM on xylene-induced inflammation

In previous studies using an acute carrageenan-induced paw edema model, Chen et al. (2008) demonstrated that both α-mangostin and γ-mangostin potently inhibit paw edema in mice. Our study employed a different model of xylene-induced ear edema to estimate the anti-inflammatory effects of CEM in mice.

Fig. 4 shows that 1.0 g/kg CEM ($n=10$) and 10 mg/kg indomethacin ($n=10$) had significant effects on ear edema compared with the control group (one-way ANOVA followed by Tukey *post hoc* test, $p<0.05$, $n=10$), with inhibition rates of 63% and 51%, respectively. The 3 g/kg CEM-treated group ($n=10$) exhibited a tendency toward aggravating xylene-induced edema, but the difference was not significant compared with the control group (one-way ANOVA followed by Tukey *post hoc* test, $p>0.05$). Previous reports indicated that the early phase of ear edema induced by xylene is mediated by histamine and serotonin, with PGs appearing to be the most important mediator in the final phase (Speroni et al., 2005). Research by Tewtrakul et al. (2009) on RAW264.7 macrophage cells showed that mangosteen extract together with α-mangostin and γ-mangostin possessed potent inhibitory effects on NO and PGE2 release. Combined with a report showing that CEM inhibits histamine release and PGE2 synthesis (Nakatani et al., 2002), we hypothesized that CEM might exert its anti-inflammatory effects by inhibiting the release of NO, histamine, and PGE2, which directly stimulate nociceptors and are involved in inflammatory pain.

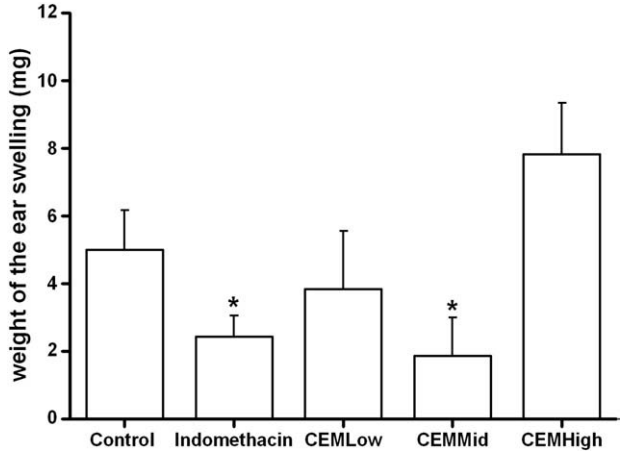

Fig. 4. Effects of CEM at different doses (CEM low, 0.5 g/kg; CEM mid, 1.0 g/kg; CEM high, 3.0 g/kg) on the weight of ear edema in xylene-induced ear inflammation ($n=10$/group). The control group received saline solution (10 mL/kg), and the reference drug was indomethacin (10 mg/kg). Error bars indicate standard deviation. $^*p<0.05$, compared with saline control group (one-way ANOVA followed by Tukey *post hoc* test).

3.4.2. Reactive oxygen species scavenging capacity of mangostins and CEM

The antioxidant effects of extracts and xanthones isolated from GML have been demonstrated using the following methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, the ferric thiocyanate method, and the 2,20-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay (Pedraza-Chaverrí et al., 2009). In present study, the different methods were employed to determine the effects of CEM and mangostins on antioxidation.

In the study of scavenging hydroxyl radicals (·OH), CEM ($n=6$), α-mangostin ($n=6$), and γ-mangostin ($n=6$) concentration-dependently enhanced ·OH scavenging capacities, with IC_{50} values of 285.1 μg/mL, 20.01 μg/mL, and 55.37 μg/mL, respectively (Fig. 5A). Chin et al. (2008) found ·OH-scavenging effects of several xanthones isolated from the fruit powder of GML. Of the 16 xanthones, only γ-mangostin exhibited ·OH-scavenging effects ($IC_{50}=0.2$ μg/mL). Pedraza-Chaverrí et al. (2009) found that α-mangostin was unable to scavenge ·OH in primary cultures of cerebellar granule neurons. In contrast, our results showed that α-mangostin exhibited significant inhibition of hydroxyl radicals in our experimental design. We further detected the effects of the compounds on scavenging superoxide radicals ($\cdot O_2^-$). Consistent with the results reported by Pedraza-Chaverrí et al. (2009), our results showed that both α-mangostin ($n=6$) and γ-mangostin ($n=6$) exhibited significant and concentration-dependent $\cdot O_2^-$ scavenging effects, with IC_{50} values of 58.63 μg/mL and 109.6 μg/mL, respectively (Fig. 5B). Thus, based on these results, we predicted that CEM might exert antioxidant effects through the main active ingredients α-mangostin, γ-mangostin, and other xanthones.

4. Discussion

G. mangostana L. is a well known fruit in south China and other south Asian countries. It has claimed efficacy for skin infections, wound infections, and diarrhea. In the present study, we isolated the xanthones α-mangostin and γ-mangostin from CEM, demonstrated their putative analgesic effects *in vivo*, and evaluated the analgesic mechanism of action of CEM, α-mangostin, and γ-mangostin.

4.1. Analgesic effect and possible mechanisms of action of CEM

The present results showed that oral administration of CEM caused an antinociceptive effect against acute nociception induced by chemical and thermal stimuli in mice. Two different analgesic methods — the acetic acid-induced writhing test and hot-plate test — were employed to identify the possible peripheral and central effects of CEM, respectively, and CEM possessed analgesic effects in both models.

Acetic acid is a widely used chemical for the evaluation of peripheral antinociceptive activity (Speroni et al., 2005). Intraperitoneal injection of acetic acid can produce peritoneal inflammation (acute peritonitis) which causes a response characterized by contraction of the abdominal muscles accompanied by extension of the forelimbs and elongation of the body. This writhing response is considered a visceral inflammatory

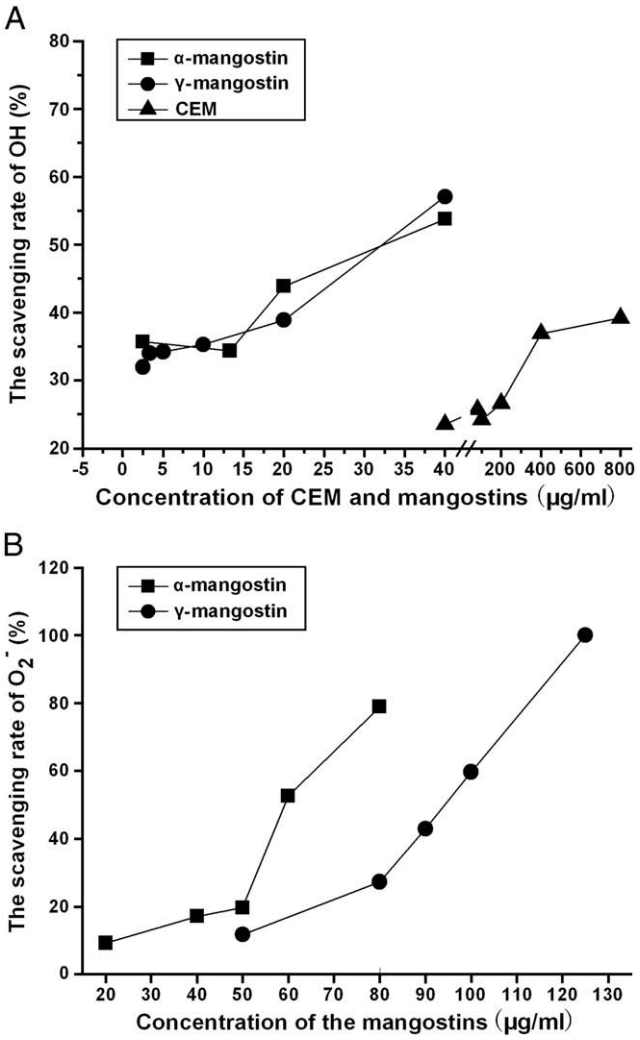

Fig. 5. Effects of CEM, α-mangostin, and γ-mangostin on (A) hydroxyl radical (·OH) scavenging activity and (B) superoxide radical ($\cdot O_2^-$) scavenging activity ($n = 6$/group).

pain model (Koster et al., 1959). In this model, pain is generated indirectly via endogenous mediators, such as bradykinin, serotonin, histamine, substance P, and PGs, which all act by stimulation of peripheral nociceptive neurons. The mechanism of the reaction to this nociceptive stimulus appears to be related to the prostanoid system (García et al., 2004; Nguemfo et al., 2007). Many analgesics, such as NSAIDs, act by inhibiting the synthesis of PG. Our results showed that CEM, at all doses tested, produced significant analgesic effects, and these effects may be attributable to PG synthesis inhibition.

To evaluate the possible central antinociceptive effects of CEM, the hot-plate test was adopted. CEM doses of 3.0, 1.0, and 0.5 g/kg significantly increased latencies in the hot-plate model compared with the control group, and these doses produced more intense effects on pain relief. These results indicate that CEM might exert its influence on pain relief through the central nervous system. The hot-plate test was used to evaluate central pain at the supraspinal and spinal levels (Marchioro et al., 2005) in which C-, Aδ type I-, and Aδ type II-sensitive fibers play a role in this model (Pietrovski et al., 2006; Lopes et al., 2009). Therefore, anti-inflammatory drugs such as indomethacin did not alleviate nociceptive behavior in the hot-plate test, and CEM possibly exerted its effects through regulating the integration of the response at the spinal cord dorsal horn or supraspinal levels.

4.2. Analgesic effect and some mechanisms of action of α- and γ-mangostins

The xanthones α-mangostin and γ-mangostin are major bioactive compounds found in the fruit hulls of GML. The hot-plate test and formalin test were chosen to evaluate the antinociceptive effects of α-mangostin and γ-mangostin. Our results demonstrated that α-mangostin and γ-mangostin are potential analgesics. The mechanisms of action of these mangostins are complex and may involve PGs, ROS, histamine, and serotonin.

Prostaglandins are lipid mediators produced by cyclooxygenases from arachidonic acid, which serve pivotal functions in inflammation and pain. In a previous study, Chen et al. (2008) found that α-mangostin and γ-mangostin significantly inhibited NO and PGE2 through inhibition of NO production and COX-2 activity, with γ-mangostin showing a greater inhibitory effect than α-mangostin (Chen et al., 2008). Inflammation is a key component of pain, and the superoxide pathway may be a key player in pain (Wang et al., 2004). Hacimuftuoglu et al. (2006) found that antioxidants led to a significant reduction in nociceptive responses in two phases of the formalin response (Hacimuftuoglu et al., 2006). Therefore, NO and PGE2 inhibition and the scavenging superoxides ·OH and $\cdot O_2^-$ may play a role in the antinociception induced by α-mangostin and γ-mangostin in the present study.

Moreover, histamine mediates a variety of physiological reactions in peripheral tissues and in the central nervous system. Several studies have demonstrated that histamine H_1 receptors play a role in physiological and pathological pain perception (Parolaro et al., 1989; Millan, 2002). Previous studies have reported that both peripheral perception and central sensitization could be attenuated in H_1 receptor knockout mice (Mobarakeh et al., 2000). α-Mangostin may serve as a histamine H_1 receptor antagonist (Chairungsrilerd et al., 1996; Iikubo et al., 2002), involving both central and peripheral pain.

Moreover, serotonin strongly potentiates nociceptive behavior when co-administered with other agents, including bradykinin, norepinephrine, and substance P (Hong and Abbott, 1994). However, its involvement in pain processing is complex because serotonin can inhibit or facilitate nociceptive transmission, depending on the nature of the applied nociceptive stimuli (Millan, 2002). Much evidence indicates that 5-HT_2 and 5-HT_3 receptors modulate nociceptive transmission. Activation of these receptors in the spinal cord produces antinociception in the formalin test and other models (Kayser et al., 2007). Among these 5-HT receptors, peripheral 5-HT_{2A} receptors have important roles in inflammatory pain (Sasaki et al., 2006), and local treatment with 5-HT_{2A} receptor antagonists suppressed second-phase flinches in the formalin test (Nakajima et al., 2009). γ-Mangostin, a selective 5-HT_{2A} receptor antagonist (Chairungsrilerd et al., 1998), produced analgesia effects on central pain (hot-plate test) and the second phase of the formalin test, as well as in peripheral pain in the first phase of the formalin test. In injured tissue, γ-mangostin likely prevents the binding of 5-HT to 5-HT_{2A} receptors at peripheral sensory terminals, inhibits the excitation of primary sensory neurons, and inhibits afferent inputs to the spinal cord (Nakanishi and Ishikawa, 2001).

Altogether, α-mangostin and γ-mangostin both possess significant analgesic effects and may be the main active constituents of CEM that affect analgesic action. The mechanisms of action involved in α-mangostin- and γ-mangostin-induced analgesia may involve inhibition of ROS and inflammatory mediators in peripheral tissue and the antagonistic effects on histamine (α-mangostin) and serotonin (γ-mangostin) receptors in the central nervous system.

4.3. Summary

We isolated the active ingredients α-mangostin and γ-mangostin from the crude ethanol extract of the fruit hulls of GML. We

demonstrated that CEM and mangostins have peripheral and central analgesic effects. The mechanism of this action may involve the production of PEG2 and superoxide. Considering that α- and γ-mangostins are antagonists of histamine H_1 and 5-HT_{2A} receptors, respectively, histamine and serotonin systems may be involved in the antinociceptive effects of α-mangostin and γ-mangostin. Our data provide support for the use of α-mangostin and γ-mangostin as analgesics in the clinic. These studies may also provide support for the use of the traditional Thai medicine GML fruit hulls in the treatment of inflammation-related diseases. Importantly, xanthones may be developed as new analgesic and anti-inflammatory drugs.

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References

Chairungsrilerd N, Furukawa KI, Ohta T, Nozoe S, Ohizumi Y. Histaminergic and serotonergic receptor blocking substances from the medicinal plant *Garcinia mangostana*. Planta Med 1996;62:471–2.

Chairungsrilerd N, Furukawa KI, Ohta T, Nozoe S, Ohizumi Y. γ-Mangostin, a novel type of 5-hydroxytryptamine 2A receptor antagonist. Naunyn Schmiedebergs Arch Pharmacol 1998;357:25–31.

Chen LG, Yang LL, Wang CC. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem Toxicol 2008;46:688–93.

Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD. Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). Phytochemistry 2008;69:754–8.

Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium* acnes. Fitoterapia 2007;78:401–8.

García MD, Fernández MA, Alvarez A, Saenz MT. Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of *Pimenta racemosa* var. ozua (Mirtaceae). J Ethnopharmacol 2004;91:69–73.

Gomez-Vargas M, Nishibayashi-Asanuma S, Asanuma M, Kondo Y, Iwata E, Ogawa N. Pergolide scavenges both hydroxyl and nitric oxide free radicals in vitro and inhibits lipid peroxidation in different regions of the rat brain. Brain Res 1998;790:202–8.

Hacimuftuoglu A, Handy CR, Goettl VM, Lin CG, Dane S, Stephens Jr RL. Antioxidants attenuate multiple phases of formalin-induced nociceptive response in mice. Behav Brain Res 2006;173:211–6.

Hong Y, Abbott FV. Behavioural effects of intraplantar injection of inflammatory mediators in the rat. Neuroscience 1994;63:827–36.

Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.

Hunskaar S, Fasmer OB, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods 1985;14:69–76.

Iikubo K, Ishikawa Y, Ando N, Umezawa K, Nishiyama S. The first direct synthesis of a-mangostin, a potent inhibitor of the acidic sphingomyelinase. Tetrahedron Lett 2002;43:291–3.

Jefferson A, Quillinan AJ, Scheinmann F, Sim KY. Isolation of γ-mangostin from *Garcinia mangostana*, and preparation of the natural mangostins by selective demethylation. Aust J Chem 1970;23:2539–43.

Kayser V, Elfassi IE, Aubel B, Melfort M, Julius D, Gingrich JA, et al. Mechanical, thermal and formalin-induced nociception is differentially altered in 5-$HT_{1A}^{-/-}$, 5-$HT_{1B}^{-/-}$, 5-$HT_{2A}^{-/-}$, 5-$HT_{3A}^{-/-}$ and 5-$HTT^{-/-}$ knock-out male mice. Pain 2007;130:235–48.

Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412–7.

Lopes LS, Pereira SS, Silva LL, Figueiredo KA, Moura BA, Almeida FRC, et al. Antinociceptive effect of topiramate in models of acute pain and diabetic neuropathy in rodents. Life Sci 2009;84:105–10.

Mahabusarakam W, Wiriyachitra P, Taylor WC. Chemical constituents of *Garcinia mangostana*. J Nat Prod 1987;50:474–8.

Mahabusarakam W, Proudfoot J, Taylor W, Croft K. Inhibition of lipoprotein oxidation by prenylated xanthones derived from mangostin. Free Radic Res 2000;33:643–59.

Marchioro M, Blank MF, Mourao RH, Antoniolli AR. Antinociceptive activity of aqueous extract of *Erythrina velutina* leaves. Fitoterapia 2005;76:637–42.

Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallal and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469–74.

Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheum Dis 2003;62:501–9.

Martindale J, Bland-Ward PA, Chessell IP. Inhibition of C-fibre mediated sensory transmission in the rat following intraplantar formalin. Neurosci Lett 2001;316:33–6.

Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.

Mobarakeh JI, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY, et al. Role of histamine H_1 receptor in pain perception: a study of the receptor gene knockout mice. Eur J Pharmacol 2000;391:81–9.

Nakajima K, Obata H, Ito N, Goto F, Saito S. The nociceptive mechanism of 5-hydroxytryptamine released into the peripheral tissue in acute inflammatory pain in rats. Eur J Pain 2009;13:441–7.

Nakanishi O, Ishikawa T. Involvement of peripheral 5-HT2A receptor activation in inflammatory pain. Nihon Rinsho 2001;59:1675–80.

Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, et al. Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. Biol Pharm Bull 2002;25:1137–41.

Nguemfo EL, Dimo T, Azebaze AG, Asongalemc EA, Alaoui K, Dongmo AB, et al. Anti-inflammatory and anti-nociceptive activities of the stem bark extracts from *Allanblackia monticola* STANER L.C. (Guttiferae). J Ethnopharmacol 2007;114:417–24.

Oliva P, Aurilio C, Massimo F, Grella A, Maione S, Grella E, et al. The antinociceptive effect of tramadol in the formalin test is mediated by the serotonergic component. Eur J Pharmacol 2002;445:179–85.

Parolaro D, Patrini G, Massi P, Mainardi P, Giagnoni G, Sala M, et al. Histamine as a central modulator of rat intestinal transit. J Pharmacol Exp Ther 1989;249:324–8.

Pedraza-Chaverrí J, Cárdenas-Rodríguez N, Orozco-Ibarra M, Pérez-Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol 2008;46:3227–39.

Pedraza-Chaverrí J, Reyes-Fermín LM, Nolasco-Amaya EG, Orozco-Ibarra M, Medina-Campos ON, González-Cuahutencos O, et al. ROS scavenging capacity and neuroprotective effect of α-mangostin against 3-nitropropionic acid in cerebellar granule neurons. Exp Toxicol Pathol 2009;61:491–501.

Peres V, Nagem TJ, de Oliveira FF. Tetraoxygenated naturally occurring xanthones. Phytochemistry 2000;55:683–710.

Pietrovski EF, Rosa KA, Facundo VA, Rios K, Marques MCA, Santos ARS. Antinociceptive properties of the ethanolic extract and of the triterpene 3β, 6β, 16β-trihidroxilup-20(29)-ene obtained from the flowers of *Combretum leprosum* in mice. Pharmacol Biochem Behav 2006;83:90–9.

Ridtitid W, Sae-Wong C, Reanmongkol W, Wongnawa M. Antinociceptive activity of the methanolic extract of *Kaempferia galangal* Linn. in experimental animals. J Ethnopharmacol 2008;118:225–30.

Sasaki M, Obata H, Kawakara K, Saito S, Goto F. Peripheral 5-HT_{2A} receptor antagonism attenuates primary thermal hyperalgesia and secondary mechanical allodynia after thermal injury in rats. Pain 2006;122:130–6.

Speroni E, Cervellati R, Innocenti G, Costa S, Guerra MC, Dall' Acqua S, et al. Anti-inflammatory, anti-nociceptive and antioxidant activities of *Balanites aegyptica* (L) Delile. J Ethnopoharmacol 2005;98:117–25.

Suksamram S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamram A. Cytotoxic prenylated xanthones from the young fruit of *Garcinia mangostana*. Chem Pharm Bull 2006;54:301–5.

Tewtrakul S, Wattanapiromsakul C, Mahabusarakam W. Effects of compounds from *Garcinia mangostana* on inflammatory mediators in RAW264.7 macrophage cells. J Ethnopharmacol 2009;121:379–82.

Wang ZQ, Porreca F, Cuzzocrea S, Galen K, Lightfoot R, Masini E, et al. A newly identified role for superoxide in inflammatory pain. J Pharmacol Exp Ther 2004;309:869–78.

Winter CA, Risely EA, Nuss GW. Carregeenin induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Exp Biol Med 1962;111:544–7.