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Anti-inflammatory and analgesic activities of the ethanolic extracts from *Zanthoxylum riedelianum* (Rutaceae) leaves and stem bark

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Abstract

We have evaluated the anti-inflammatory and analgesic properties of the leaves (LCE) and stem bark (BCE) crude extracts of *Zanthoxylum riedelianum* (Rutaceae). Different fractions of the stem bark extract (hexane, BCEH; dichloromethane, BCED; ethyl acetate, BCEE; and lyophilized aqueous residual, BCEW) were also investigated. We studied the effects of the extracts and fractions using the rat paw oedema test induced by carrageenan, dextran, histamine or nystatin; the mouse abdominal constriction test; the mouse hot-plate test (only for LCE and BCE); and the mouse formalin test. Both extracts and all BCE fractions displayed anti-inflammatory activity in the carrageenan-induced oedema model, but not for dextran, histamine or nystatin. Considering the analgesic models, both extracts showed antinociceptive activity, but BCE was more active than LCE in models of central pain. All BCE fractions showed significant inhibition in the abdominal constriction test and in both phases of the formalin test. When BCED was submitted to phytochemical procedures it led to the isolation of six lignans (sesamin, methylpluviatolide, dimethylmatairesinol, piperitol-4′-*O*-γ,γ-dimethylallyl ether, kaerophyllin and hinokinin), and a triterpene (lupeol). Inhibition of cyclooxygenase and its metabolites may have been involved in the mechanism of action of this plant, considering previous studies reporting the anti-inflammatory and analgesic activity for the identified lignans, as well as anti-inflammatory activity for lupeol.

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Introduction

Zanthoxylum riedelianum Engl. (Rutaceae) is a wild Brazilian tree 8–18 m high known as "mamica-de-porca" due to the aculeus shape of its thorns, and it has been used as an analgesic for tooth pain (Pio Correa 1974). Only one report has been found describing the chemical composition of the essential oil from *Z. riedelianum* (Guy et al 2001). The genus *Zanthoxylum* comprises 250 species. Coumarins, flavonoids, alkaloids, steroids, terpenoids and lignans isolated from *Z. rhoifoliumi, Z. piperitum, Z. americanum, Z. naranjillo* and others have been described. Reports have shown anti-inflammatory and analgesic biological activity (Bastos 1991; Rahman et al 2002), and anticancer (Tin-Wa et al 1974; Bowen & Lewis 1978), antibacterial (Moura et al 1998; Gonzaga et al 2003a, b), antifungal (Diéguez-Hurtado et al 2003) activity. It has been used as a digestive tonic (Perry 1980; Xie & Huang 1984; Ghani 1998), a monoaminoxidase inhibitor (Jo et al 2002), and in anti-platelet aggregation (Chen et al 1995; Tsai et al 2000).

Based on the chemotaxonomy of *Zanthoxylum* genus, we have evaluated the anti-inflammatory and analgesic properties of the leaves (LCE) and stem bark (BCE) crude ethanolic extracts and fractions (hexane, dichloromethane, ethyl acetate and water lyophilized fractions) in pre-clinical models.

Materials and Methods

Plant material

Zanthoxylum riedelianum Engl. (Rutaceae) leaves and stem bark were collected at São Simão (São Paulo, SP, Brazil). Plant identity was confirmed by Dr José Rubens Pirani from

the Botany Institute Herbarium of São Paulo University. A voucher specimen was deposited at the herbarium of the Faculdade de Filosofia, Ciências e Letras of São Paulo University at Ribeirão Preto (SPFR 1371). The material was air dried at 40°C and then powdered.

General procedures

NMR spectra were recorded on a Brucker ARX 400 spectrometer. Vacuum-liquid chromatography (VLC) was carried out with silica gel Merck 9385, 40–63 μm 60 H 230–400 mesh ASTM (Merck Co, Darmstadt, Germany) in glass columns with 2–5 cm i.d. Preparative thin layer chromatography (TLC; silica gel $PF_{254+366}$, Merck) semi-preparative HPLC separations were carried out on a Shimadzu SCL-10 AVP liquid chromatography system equipped with a SPD-M10AVP Shimadzu UV-DAD detector (the channel was set at 255 nm), and column (ODS, 250×4.6 mm, 5 μm). Dichloromethane was acquired from Acros Co., New Jersey. Ethyl acetate, hexanes and methanol were supplied by Mallinckrodt Co., Xalostoc, Mexico. Ethanol was bought from a local distillery and purified by distillation. Indometacin (Prodome, Campinas, Brazil), kappa carrageenan type III (Iota-Fluka-Biochemika Co., St Louis, MA), cyproheptadine and histamine (Sigma Co., St Louis, MA), sodium chloride and acetic acid (Merk Co. Darmstadt, Germany), dextran (MW 70000 Da, Pharmacia, Milan, Italy), nystatin 8.5% (Bristol-Myers-Squibb, Princeton, USA), Tween 80 (Labynth Co, Diadema SP, Brazil) and morphine (Cristalia Co., Itapira, Brazil) were acquired from the market.

Extraction and partition procedures

Powdered leaves (1.62 kg) and stem bark (2.12 kg) were exhaustively extracted with ethanol (99.5%) by maceration followed by percolation at room temperature. The extracts were named as leaf crude extract (LCE; 44.8 g, yield 2.8%) and bark crude extract (BCE; 37.1 g, yield 1.8%). The crude BCE extract (20 g) was dissolved in MeOH:H_2O (7:3) and submitted to sequential partition with hexanes (BCEH; yield 21 g), dichloromethane (BCED; yield 10 g), or ethyl acetate (BCEE; yield 1 g), and the remaining aqueous layer was lyophilized (BCEW; yield 5 g). Solvents were evaporated under reduced pressure to yield crude extracts and fractions, which were subsequently used for the pharmacological tests.

Isolation and identification of compounds

The dichloromethane fraction (BCED; 5 g) was chromatographed over silica gel under a VLC system (isocratic; ethyl acetate) to yield 32 fractions (10 mL each). Fractions 1 and 2 were combined (352 mg) and crystallized in methanol affording triterpene **1** (29 mg). Fractions 3 to 8 (1.5 g) were assembled after TLC analysis and re-submitted to VLC using gradient elution starting with hexane (fractions 1′ to 5′), followed by hexane:ethyl acetate 4:1 (fractions 6′ to 17′), and hexane:ethyl acetate 1:1 (fractions 18′ to 30′). Fractions 11′ to 14′ (56 mg) were combined furnishing compound **2** (7 mg) by preparative TLC using toluene:ethyl acetate 7:3 as mobile phase. Fraction 15′ (29 mg) was submitted to semi-preparative reverse-phase HPLC purification using methanol:H_2O (75:25) as mobile phase furnishing compounds **3** (4 mg) and **4** (3 mg), as well as fractions 16′ to 18′ (95 mg), which were combined furnishing compounds **5** (58 mg) and **6** (4 mg), respectively. Fractions 22′ to 23′ (113 mg) were also combined and submitted to crystallization using MeOH, affording compound **7** (52 mg) (Figure 1A).

Pharmacological assays

Animals

Wistar male rats (*Rattus norvergicus*; 180–200 g) and Swiss male albino mice (*Mus musculus*; 20–25 g) were used. Animals were acquired from the Central Biotery of the São Paulo University at Ribeirão Preto, and were kept in polyethylene boxes (n = 5) under controlled circadian cycle and temperature (22 ± 2°C), with free access to food and water. The study was authorized by the Ethical Committee for Animal Care of the University of São Paulo (Process number 05.1.783.53.2), in accordance with the Federal Government legislation on animal care.

Treatments

Animals received the extracts and reference drugs orally, 1 h before the administration of an inflammatory agent. The extracts were administered at the doses of 50, 100 and 250 mg kg^{-1}, respectively, and BCE fractions were administered at 100 mg kg^{-1}. Control group received 0.5 mL distilled water with 3% Tween 80. The reference drugs used, indometacin (10 mg kg^{-1}) and cyproheptadine (10 mg kg^{-1}), were administered orally. Morphine was administered intraperitoneally (10 mg kg^{-1}) 30 min before injection of formalin.

Anti-inflammatory activity

Rat paw oedema

Four different inflammatory agents were used to induce rat paw oedema: carrageenan (1000 μg/paw), dextran (100 μg/paw), histamine (50 μg/paw) and nystatin (47600 units/paw), which were injected into the right-hind-paw plantar surface (Winter et al 1962). The animals were treated with indometacin for carrageenan and nystatin models, and cyproheptadine for dextran- and histamine-induced oedema. The inflammatory agents were dissolved in sterile saline solution 0.9% (0.1 mL/paw). To act as the control, animals received saline solution into the left paw. The measurement of foot volumes was carried out following the plethysmographic method described by Ferreira (1979), using Ugo Basile plethysmometer model 7140 (Varese, Italy).

Analgesic activity

Abdominal constriction test

The test was performed according to Koster et al (1959), using groups of male Swiss mice (n = 10). After each treatment, animals were injected intraperitoneally with 0.25 mL 1% acetic acid solution, and stretching episodes were recorded within 5-min intervals for 20 min. Indometacin was used as reference drug (10 mg kg^{-1}, p.o.).

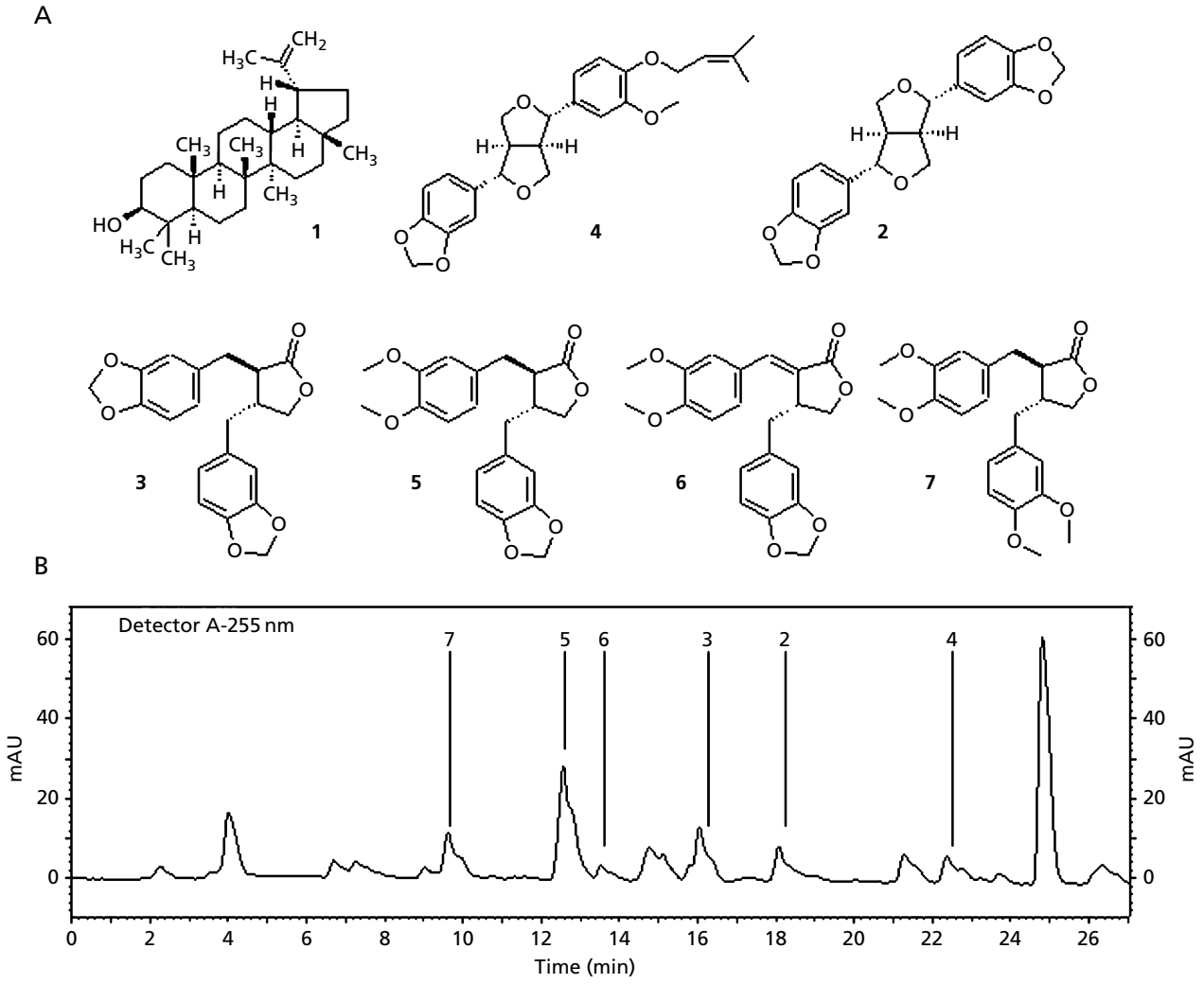

Figure 1 A. Isolated and identified compounds: lupeol (**1**), sesamin (**2**), hinokinin (**3**), piperitol-4′-O-γ, γ-dimethylallyl ether (**4**), methylpluviatolide (**5**), kaerophyllin (**6**), and dimethylmatairesinol (**7**). B. HPLC chromatographic profile of the dichloromethane fraction from *Z. riedelianum* bark crude extract by using gradient elution consisted of MeOH:H_2O 9:1 to MeOH in 30 min, 1 mL min^{-1}, in a C_{18} ODS 5 μm, 250 × 4.6 mm column.

Hot-plate test

The hot-plate assay was performed according to Eddy & Leimbach (1953), which involved exposing mice to a hot surface. The hot plate apparatus (Ugo Basile DS37, Italy) was maintained at 55 ± 0.5°C. Animals were individually exposed, and the time they spent licking the footpad or any paw (latency time) was recorded. The cut-off time was set at 20 s. The measurements were performed at 0, 30, 60, 90 and 120 min after treatment. BCE fractions were not tested by this method. Morphine (10.0 mg kg^{-1}, i.p.) was used as the reference drug.

Formalin test

This test was performed with mice. The animals received 20 μL 2.5% formalin solution in their right footpad, according to Hunskaar & Hole (1987). After formalin administration, animals were isolated and observed for the first 5 min (early phase – neurogenic pain) and between 20–25 min (late phase – inflammatory pain). Treatments were undertaken by administration of 150 mg kg^{-1} LCE or BCE crude extracts, or 100 mg kg^{-1} BCE fractions. Morphine and indometacin (10 mg kg^{-1}) were used as the reference drugs, respectively.

Statistical analysis

Values are presented as mean ± s.e.m. (standard error of mean). The level of statistical significance was determined by analysis of variance followed by Tukey–Kramer multiple comparison tests to compare treated groups with control. Levels of 95% of interval confidence or higher were considered significant. Statistical significance was shown as $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$.

Results

Isolation and identification of compounds

The phytochemical study of BCED led to the isolation of seven compounds (Figure 1B), and its HPLC chromatographic profile is displayed in Figure 1B. The chemical structures of all isolated compounds (Figure 1A) were established by UV-vis, ^{1}H and ^{13}C NMR data analysis in comparison with the literature, as follows: lupeol (**1**), sesamin (**2**), hinokinin (**3**), piperitol-4′-O-γ,γ-dimethylallyl ether (**4**), methylpluviatolide (**5**), kaerophyllin (**6**) and dimethylmatairesinol (**7**) (Abe et al 1974; Pelter et al 1976; Rucker & Langmann 1978; Mikaya et al 1981; Lopes et al 1983; Agrawal & Thakur 1985; Koul et al 1988; González et al 1990; Bastos 1996; Chang et al 2000; Heleno et al 2006).

Rat paw oedema

The oral administration of both extracts inhibited significantly, in a dose-dependent manner, the carrageenan-induced oedema. LCE was able to reduce the oedema formation at 2 and 3 h, when administered at 100 mg kg^{-1} ($P<0.05$) and 250 mg kg^{-1} ($P<0.01$ and $P<0.001$). All doses of BCE and its fractions inhibited significantly the carrageenan oedema at 2 and 3 h. BCED was more active, reducing the oedema formation by 62, 63 and 57% ($P<0.001$) after 2, 3 and 4 h, respectively (Table 1). The results were statistically significant in comparison with the control.

Neither extracts nor the BCE fractions showed any significant results towards rat paw oedema induced by dextran, histamine or nystatin (data not shown).

Abdominal constriction test

Extracts showed significant inhibition of peripheral pain in a dose-dependent manner. Both extracts at doses of 100 and 250 mg kg^{-1} produced significant inhibition for all observed times. All doses of BCE administered reduced abdominal constrictions ($P<0.001$) at 5 min. However, BCE 50 mg kg^{-1} did not reduce significantly the constrictions at 10, 15 and 20 min. With regard to the BCE fractions, only BCED and BCEE displayed significant activity at $P<0.05$ and $P<0.01$, respectively. Also, LCE at the evaluated doses reduced significantly the abdominal constrictions in mice by 86% ($P<0.001$) at 15 and 20 min (Table 2).

Hot-plate test

Only when LCE was administered at 250 mg kg^{-1} was a significant increase ($P<0.001$) seen in latency time at all observations. Unlike LCE, BCE administration was more active and showed a dose-dependent response (Table 3). When BCE was administered at 50 mg kg^{-1}, significant activity was observed at 30 ($P<0.01$), 60 ($P<0.001$) and 90 ($P<0.01$) min. BCE 250 mg kg^{-1} increased the latency time significantly ($P<0.01$). The animals treated with morphine gave a response latency period longer than 20 s, since it was established as the cut-off time for the protocol.

Formalin test

Both extracts and BCE fractions were able to reduce the licking time at the second phase ($P<0.001$). During the first phase only BCE, BCED and BCEE showed statistically significant activity ($P<0.001$; Table 4).

Discussion

This study has shown that both extracts displayed anti-inflammatory, peripheral and central analgesic activity. Carrageenan-induced oedema is a classical pharmacological model in the study of non-steroidal anti-inflammatory drugs (Bispo et al 2001). Subcutaneous injection of carrageenan into rat hind paw produced inflammation with three

Table 1 Effect of *Z. riedelianum* extracts, its fractions and indometacin treatments on carrageenan-induced rat paw oedema

Groups	Dose (mg kg^{-1})	Hour 2		Hour 3		Hour 4	
		Oedema (mL)	% Inhibition	Oedema (mL)	Inhibition (%)	Oedema (mL)	% Inhibition
Control	–	0.193 ± 0.03	–	0.205 ± 0.06	–	0.155 ± 0.03	–
Indometacin	10	0.103 ± 0.01	46.6*	0.122 ± 0.03	41.0*	0.088 ± 0.01	43.2*
LCE	50	0.118 ± 0.02	38.9	0.134 ± 0.03	30.6	0.112 ± 0.02	27.8
	100	0.110 ± 0.02	43.0*	0.122 ± 0.01	40.5*	0.098 ± 0.02	36.8
	250	0.094 ± 0.02	51.3**	0.080 ± 0.03	58.6***	0.088 ± 0.02	43.2*
BCE	50	0.100 ± 0.002	48.2*	0.122 ± 0.03	45.4**	0.104 ± 0.01	32.9
	100	0.084 ± 0.01	56.5**	0.084 ± 0.02	59.0***	0.080 ± 0.01	48.4**
	250	0.086 ± 0.01	55.4**	0.080 ± 0.02	61.0***	0.078 ± 0.01	49.7**
BCEH	100	0.104 ± 0.01	46.1*	0.096 ± 0.02	53.2***	0.098 ± 0.02	36.8
BCED	100	0.074 ± 0.01	61.7***	0.076 ± 0.02	62.9***	0.066 ± 0.01	57.4***
BCEE	100	0.086 ± 0.02	55.4**	0.122 ± 0.02	45.4**	0.096 ± 0.03	38.1
BCEW	100	0.094 ± 0.01	51.3**	0.104 ± 0.03	63.3***	0.080 ± 0.01	48.4**

Results are shown as mean oedema volume ± s.e.m. and inhibition percentage when compared with control group. Statistical significance according to Tukey–Kramer test. *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

Table 2 Effect of *Z. riedelianum* extracts, its fractions and indometacin treatments on acetic acid abdominal constriction test in mice

Groups	Dose (mg kg^{-1})	Intervals (number of abdominal constrictions)			
		5 min	10 min	15 min	20 min
Control	–	12.7 ± 1.3	18.0 ± 2.9	10.3 ± 1.0	7.2 ± 0.7
Indometacin	10	2.0 ± 0.4***	2.6 ± 1.2***	3.8 ± 0.8***	2.4 ± 0.3***
LCE	50	3.9 ± 1.2***	7.1 ± 1.6***	3.9 ± 1.0***	2.4 ± 0.6***
	100	3.0 ± 1.3***	6.0 ± 1.7***	3.6 ± 0.9***	2.3 ± 0.7***
	250	2.9 ± 0.7***	4.4 ± 1.5***	1.4 ± 0.5***	1.0 ± 0.4***
BCE	50	5.8 ± 0.9***	12.5 ± 1.5	7.4 ± 1.3	5.1 ± 1.1
	100	4.1 ± 1.0***	7.3 ± 0.7**	4.0 ± 0.7**	2.8 ± 0.6**
	250	2.7 ± 0.6***	4.0 ± 1.0***	2.4 ± 0.7***	1.2 ± 0.5***
BCEH	100	4.0 ± 0.9***	8.3 ± 1.6*	4.6 ± 0.4**	3.4 ± 0.6*
BCED	100	5.1 ± 0.8***	4.6 ± 0.5***	3.3 ± 0.9***	1.6 ± 0.5***
BCEE	100	2.4 ± 0.7***	4.8 ± 0.9***	3.9 ± 0.6***	2.0 ± 0.5***
BCEW	100	5.0 ± 1.0***	11.8 ± 2.0	5.5 ± 1.0*	3.4 ± 0.5*

Results are shown as mean number of abdominal constrictions ± s.e.m. Statistical significance according to Tukey–Kramer test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 3 Effect of *Z. riedelianum* extracts treatments on hot-plate test in mice

Groups	Dose (mg kg^{-1})	Intervals (time in s)			
		30 min	60 min	90 min	120 min
Control	–	3.0 ± 0.4	4.7 ± 0.6	5.5 ± 0.5	4.4 ± 0.7
Morphine	10	> 20***	> 20***	> 20***	> 20***
LCE	50	3.8 ± 0.4	6.2 ± 0.8	10.0 ± 1.3	9.7 ± 1.5
	100	5.0 ± 0.6	9.2 ± 1.2*	10.8 ± 1.4	12.7 ± 1.4
	250	9.2 ± 1.2***	14.2 ± 1.4***	14.4 ± 1.5***	12.9 ± 2.1*
BCE	50	8.4 ± 0.8**	12.3 ± 1.1***	12.0 ± 1.2**	11.34 ± 1.6
	100	8.5 ± 1.2**	12.0 ± 1.0***	14.3 ± 1.3***	12.9 ± 0.8*
	250	9.8 ± 1.7***	16.1 ± 1.1***	15.5 ± 1.7***	16.3 ± 1.2***

Results are shown as mean of latency time ± s.e.m. Statistical significance according to Tukey–Kramer test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 4 Effect of treatments on formalin test

Groups	Dose (mg kg^{-1})	First phase (0–5 min)		Second phase (20–25 min)	
		Licking time (s)	% inhibition	Licking time	% inhibition
Control	–	68 ± 3.3	–	89 ± 4.0	–
Morphine	10	10 ± 2.8	84.9***	1 ± 0.2	89.7***
Indometacin	10	57 ± 3.3	16.2	23 ± 0.9	74.2***
LCE	150	59 ± 2.1	13.1	28 ± 3.4	68.1***
BCE	150	40 ± 3.3	41.4***	21 ± 1.2	76.8***
BCEH	100	54 ± 2.6	20.6	47 ± 3.7	46.8***
BCED	100	33 ± 3.5	50.9***	38 ± 2.7	56.8***
BCEE	100	48 ± 2.5	29.4***	42 ± 2.0	52.9***
BCEW	100	55 ± 4.3	18.8	35 ± 3.3	59.8***

Results were expressed as mean of time expended licking hind paw (s) ± s.e.m. and inhibition percentage when compared with control group. Statistical significance according to Tukey–Kramer test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

characteristic phases (Okpo et al 2001). The first phase (first 90 min) was attributed to histamine and serotonin release from perivenular mast cells and activation of plasmatic proteins. The second phase (90–150 min) was characterized by kinin system activation and bradykinin release. After 150 min, when the third phase was installed, cyclooxygenase (COX) metabolites were released, presenting mainly high amounts of prostaglandin E_2 (PGE_2) (Di Rosa et al 1971). At 2 and 3 h, LCE (250 mg kg^{-1}) and all BCE doses and its fractions inhibited the oedema, showing higher activity than the indometacin-treated group. Only BCE (100 and 250 mg kg^{-1}), BCED and BCEW showed inhibitory activity at 4 h.

Dextran induces oedema formation by mast cell activation, releasing high amounts of biologically active amines, such as histamine and serotonin. Non-steroidal anti-inflammatory agents are not able to inhibit this kind of inflammatory process (Merlos et al 1990). Histamine administration in-loco caused an endothelial constriction by H_1 binding, resulting in an increase of plasma exudation (Carvalho 1998). The extracts did not show ability to inhibit the inflammatory process induced by biologically active amines. Nystatin promotes alteration on lysosomal membranes, which causes proteolytic enzyme release (Schiatti et al 1970; Niemegeers et al 1975). This is a subchronic inflammatory model. Neither of the extracts nor the BCE fractions were able to reduce oedema induced by dextran, histamine or nystatin.

Although acetic acid-induced abdominal contractions represent a peripheral nociception model (Wei et al 1986), this is not a specific model, since several compounds such as tricyclic antidepressants (Takahashi & Paz 1987) and antihistaminergics (Yeh 1985) decrease abdominal constrictions. Acetic acid promotes a visceral pain model, sensitive to non-steroidal anti-inflammatory agents. Arachidonic acid derivatives produced by COX activity, mainly prostaglandins, promote pain by decreasing the excitation threshold of pain neurons (Franzotti et al 2000). Intraperitoneal administration of acetic acid caused irritation of serous membranes provoking stereotyped behaviour, characterized by abdominal contractions (Le Bars et al 2001). The quantification of prostaglandins in peritoneal exudates after intraperitoneal injection of acetic acid demonstrated high levels of $PGE_{2\alpha}$ and $PGF_{2\alpha}$ in the 30 min after stimulus (Deraedt et al 1980). Both extracts showed significant inhibition in the peripheral analgesic model, demonstrated by the abdominal constriction test. All LCE administered doses were able to inhibit the constrictions at all times. On the other hand, BCE only inhibited the mouse constrictions at 100 and 250 mg kg^{-1}, and the inhibition was significantly lower than that observed for LCE. However, all BCE fractions displayed activity in this assay, with the BCED displaying pronounced activity.

The formalin test is considered a valid model for clinical pain (Tjolsen et al 1992). In this test, the first phase or acute phase (0–5 min) has been thought to result from direct activation of nociceptive afferent fibres. The second or tonic phase (20–25 min) is an inflammatory peripheral process (Coderre & Melzack 1992; Abbadie et al 1997). Drugs which mainly act centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripheral analgesics only inhibit the second phase (Santos et al 1997). BCE administration, like morphine, inhibited both phases of the formalin test, whilst LCE administration reduced only the second phase.

The hot-plate test is commonly used for assaying narcotic-like analgesics. However, other drugs such as sedatives, muscle relaxants or psychomimetic drugs may show positive activity (Vaz et al 1996). The hot-plate test is characterized by supra-spinal organized answers that involve brain functions to pain stimulus (Gardmark et al 1998). BCE showed significant activity at all administered doses, which was comparable with the formalin results, and LCE displayed significant activity only at 250 mg kg^{-1}.

According to the results, it is suggested that both extracts might display anti-inflammatory activity associated with COX inhibition, as it has been observed for other species belonging to the same genus, as well as for some of the identified lignans. The two extracts displayed different analgesic actions, a peripheral action was observed for LCE and a central action for BCE. Anti-inflammatory action seems to be more pronounced to BCE, especially BCED, from which the lignan compounds were isolated. Lupeol, sesamin and hinokinin have been reported to display significant anti-inflammatory activity. Lupeol was reported as an anti-inflammatory due to its ability to inhibit carrageenan oedema (Fernandez et al 2001a), as well as PGE_2 and tumour-necrosis factor-α in-vitro (Fernandez et al 2001b). However, lupeol has not been reported to exert analgesic, anti-pyretic or ulcerogenic activity (Geetha & Varalakshmi 2001). Sesamin has displayed, among other activities, a role of actions in the inflammatory process by inhibiting primary activation pathways, such as mitogen-activated protein kinase and nuclear factor kappa B (Hou et al 2003; Jeng et al 2005). In addition, it was not able to inhibit PGE_2 in-vitro (Lee et al 2005). Hinokinin, a dibenzylbutirolactone lignan, has been reported to display anti-inflammatory, peripheral analgesic (da Silva et al 2005), trypanocidal (Saraiva et al 2007), cytotoxicity against P-388 and HT-29 tumour cell lines (Lin et al 2004) and anti-human hepatitis B virus-anti-HBeAg activity (Huang et al 2003). Other compounds such as dimethylmatairesinol have been reported as cytotoxic to three different human tumour cells (Chang et al 2000). Piperitol-4′-*O*-γ, γ-dimethylallylether, kaerophyllin, and methylpluviatolide, also isolated from *Z. naranjilo*, have displayed none, moderate and high activity against *Trypanosoma cruzi*, respectively (Bastos et al 1999). Despite this, it is suggested that the observed central analgesic effect was due neither to lignans nor to triterpenes.

Conclusions

BCE and LCE displayed anti-inflammatory and analgesic activity. The mechanism of action was not determined, but our results suggested that COX and its metabolites might have been involved in the anti-inflammatory and peripheral analgesic effects. Phytochemical investigation of the dichloromethane fraction of the crude stem bark hydroalcoholic extract allowed the identification of lignans and triterpenes.

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