Study of Antinociceptive Effect of Isolated Fractions from *Petiveria alliacea* L. (tipi) in Mice

Patrícia Bezerra Gomes,[a] Maria Mirele da Silva Oliveira,[a] Carlos Renato Alves Nogueira,[a] Emmanuelle Coelho Noronha,[a] Lyvia Maria Vasconcelos Carneiro,[a] José Noberto Sousa Bezerra,[b] Manoel Andrade Neto,[b] Silvania Maria Mendes Vasconcelos,[a] Marta Maria França Fonteles,[c] Glauce Socorro Barros Viana,[a] and Francisca Clea Florenço de Sousa*,[a]

[a] *Department of Physiology and Pharmacology, Federal University of Ceará;* [b] *Department of Organic Chemistry, Federal University of Ceará; and* [c] *Department of Pharmacy, Federal University of Ceará; Rua Cel. Nunes de Melo 1127, 60430–270, Fortaleza, Ceará, Brazil.* Received May 17, 2004; accepted September 30, 2004

The acetate (FA), hexanic (FH), hydroalcoholic (FHA) and precipitated hydroalcoholic (FHAppt) fractions from the root of *Petiveria alliacea* L. were evaluated for antinociceptive effect using the abdominal constriction induced by acetic acid, hot-plate, formalin tests. The open field and rota rod tests were used to evaluate psychomotor function and myorelaxant activity. The fractions were administered intraperitoneally in mice at doses of 100 and 200 mg/kg. Inhibitions of abdominal constrictions were observed with all doses of the fractions, as compared to control. FH and FHAppt, at both doses, reduced the nociception produced by formalin in the 1st (0—5 min) and 2nd (20—25 min) phases, however FHA (100, 200 mg/kg) and FA 200 mg/kg presented significant inhibition on the 1st and 2nd phases, respectively, of this test. A reduction of the locomotor activity was observed in the open field test with all the fractions. These fractions failed to affect the motor coordination in the rota rod test. Results showed that the different fractions of *Petiveria alliacea* L. have different antinociceptive potentials as demonstrated in the experimental models of nociception in mice, supporting folk medicine use of this plant.

Key words *Petiveria alliacea*; antinociceptive effect; abdominal constriction; hot-plate; formalin test

Petiveria alliacea L. (tipi), a shrub from Phytolaccaceae family is a perennial, subligneous, upstanding herbaceous, with characteristic garlicky odour and slender, compressed, semi-erect, mounting branches. Leaves present short petioles and are alternate, membranous, entire, sharp or acuminating at the apex and narrowing at the base. Flowers are sessile, reduced and on lean bracteate ears. The fruit is capsular, reduced and cuneiform.[1,2)]

Indigenous to the Amazon Rainforest and widely distributed in other areas including tropical America, the Caribbean, Africa, Sri Lanka, and the south-eastern Unites States.[3,4)] It was brought by slaves to Brazil where it is popularly known as tipi, pipi, guine root, erva-pipi, anamu, apacin, garlic guinea henweed.[3,5)] This plant is commonly used for several medicinal purposes. The roots in decoction or powder and the infusion of leaves, is employed as antispasmodic, antirheumatic (topic use), anti-inflammatory,[6)] antinociceptive,[7)] hypoglycemiant and abortifacient.[8,9)] They are reputed as sudorific, anti-venereal, diuretic, sedative, antihelminthic, emmenagogue, stimulant, anesthetic and depurative.[8—10)]

Tipi was used in religious ceremony by slaves, who called the herb "to tame the master" a reference to its toxic and sedative properties. The chief pharmacologic activities of tipi (already identified in a preliminary report) relates to the areas of infectology, rheumatology and oncology.

This plant contains a diversity of biologically active compounds such as essential oil (Petiverina), saponinic glicosides, isoarborinol-triterpene, isoarborinol-acetate, isoarborinol-cinnamate, steroids, alkaloids, flavonoids and tannins.[11—13)] Acoording to the literature, the tipi root chemical analysis have revealed coumarins, benzyl-hydroxy-ethyl-trisulfide, benzaldehyde, benzoic acid, dibenzyl trisulphide, potassium nitrate, b-sitosterol, isoarborinol, isoarborinol acetate, isoarborinol cinnamate, polyphenols, trithiolaniacine, glucose and glycine.[8)]

The recently reported studies of Benevides *et al*.,[14)] who isolated di-*n*-propyl disulfide, benzyl hydroxymethyl sulfide and several other antifungal polysulfides from the roots of *Petiveria alliacea* L., as well as the work of Szczepanski *et al*. [12)]

Dibenzyl trisulphide, a main lipophilic compound in *Petiveria alliacea* L., has interesting biological activities, affecting, in addition to immunomodulation, microtubule-dependent cellular events and tyrosine phosphorylation-mediated MAP kinase signalling.[15,16)]

Petiveria alliacea L. is included in the Brazilian and Paraguay Pharmacopoeias and by the Japanese Directory of Drugs.

The purpose of the present study was to evaluate the antinociceptive effect of the acetate (FA), hexanic (FH), hydroalcoholic (FHA) and precipitated hydroalcoholic (FHAppt) fractions of *Petiveria alliacea* L. in different experimental models of nociception in mice.

MATERIALS AND METHODS

Plant Material The roots of tipi were collected in Pentecoste, state of Ceará, and brought to the Department of Organic Chemistry of the Federal University of Ceará (Brazil). The exsicatae was deposited at Prisco Bezerra Herbarium under the number 30.111.

Preparation of the Fractions The roots of tipi were dried up protected from the sun, reduced to powder and then extracted exhaustively at room temperature with hydroalcoholic solution (ethanol/water, 50% v/v). The resulting solu-

* To whom correspondence should be addressed. e-mail: clea@ufc.br

tion was filtered and partitioned between hexane successively (four times). A volume of solvent corresponding to 25% of the total solution volume was used. The procedure was repeated in relation to ethyl acetate. Hexane and ethyl acetate solutions were evaporated using a rotary evaporator and the fractions (FH and FA) were obtained. From the resulting hydroalcoholic solution, submitted to water-bathing evaporation (70 °C), a precipitate (FHAppt) was obtained and the remaining material extracted (FHA). FA, FH, FHA and FHAppt (100, 200 mg/kg, i.p.) were dissolved completely with distilled water while were emulsificated in water with 3% Tween 80.

Animals Female Swiss mice (25—30 g), 8—10 per group, were used for the antinociceptive activity tests. The animals were housed in standard environmental conditions (22±1 °C, humidity 60±5%, 12 h light : 12 h dark cycle) with free access to a standard commercial diet and water *ad libitum* following international recommendations (Canadian Council of Animal Care, 1993). All experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services. The study protocol was approved by the local "Animal Ethics Committee".

Antinociceptive and Behavioral Tests. Acetic Acid Induced Nociception (Writhing Test) The method of Koster *et al*.[17] was utilized. Animals were injected with 0.6% acetic acid (10 ml/kg, i.p.) and the number of writhings during 20 min was registered. Animals were treated with the fractions, 30 min (i.p.) before the acetic acid injection. Indomethacin (2 mg/kg, i.p.) was used as the reference drug. Control animals received vehicle (10 ml/kg of 3% solution of Tween 80). The antinociceptive effect was expressed as percentage of inhibition of the abdominal constrictions.

Formalin Test The method of Hunskaar *et al*.[18] was used. Formalin 1% (20 μl) was administered to mice by intraplantar route in the right hind paw. The animals were observed to evaluate the reaction to pain (licking time) during the first phase, neurogenic (0—5 min), and the second phase, inflammatory (20—25 min). Mice were pretreated with naloxone (Nal) 2 mg/kg, s.c. and, after 15 min the animals received the FA, FH, FHA and FHAppt (200 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) or vehicle (10ml/kg of 3% solution of Tween 80); 30 min later, they received formalin. Morphine was used as standard drug.

Thermal Nociception (Hot-Plate) The hot-plate test was used to measure response latencies. Animals were submitted at the pre-test in a Ugo Basile hot-plate with a constant temperature of 55±1 °C. The animal that showed the reaction time at the thermal stimulus (jump or lick the hind paw) higher than 20 s was discarded. The reaction time was registred before and 30, 60, 90 min.[19] after the administration of the fractions and control animals received vehicle (10 ml/kg of 3 % solution of Tween 80), with cut-off time of 40 s to avoid animal paw lesion; morphine (10 mg/kg, i.p.) was used as reference drug.[19] In order to examine the involvement of opioid mechanism in the response to the FHAppt (100 and 200 mg/kg, i.p.), naloxone (2 mg/kg, s.c.) was administered 15 min prior to the fraction (FHAppt) or morphine (10 mg/kg, i.p.) injections.

Open Field The open field area was made of acrylic (transparent walls and black floor, 30×30×15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploration activity of the animal.[20] Animals were treated with the fractions. After 30 min of administration each mouse was placed in the center of the arena and the number of squares crossed, with the four paws, (locomotor activity) was recorded for 5 min. Diazepam (2 mg/kg, i.p.) was used as the reference drug. Control animals received vehicle (10 ml/kg of 3% solution of Tween 80).

Rota Rod For the rota rod test, the animals were treated with the fractions and 30 min of administration each animal was placed with the four paws on a 2.5 cm diameter bar, 25 cm above the floor, which was turning at 12 rpm. For each animal, the number of falls (up to three falls) and the time of permanence on the bar for 1 min were registered.[21] Diazepam (2 mg/kg, i.p.) was used as the reference drug. Control animals received vehicle (10 ml/kg of 3% solution of Tween 80).

Statistical Analysis All results were expressed as mean±S.E.M. and the statistical significance was determined using analysis of variance ANOVA followed by Student–Newman–Keuls test. Values were considered significantly different at $p<0.05$.

RESULTS

The results obtained with the abdominal constriction test are shown in Table 1. The intraperitoneal administration of *Petiveria alliacea* L. (100, 200 mg/kg) had a significant effect on the number of abdominal constrictions induced by acetic acid, causing a 77.4—96.2% inhibition, as compared with control. Indomethacin (2 mg/kg, i.p.) used as the standard drug caused an inhibition of 97.3%. All of the fractions caused significant antinociception in both doses on abdominal constrictions test, and most effective was FHAppt.

In formalin test (Table 2), FH (100 and 200 mg/kg), FHAppt (100, 200 mg/kg) and FHA (100 and 200 mg/kg) demonstrated antinociceptive activity producing effective blockade of the first phase (0—5 min) causing a 51.4, 55.4, 28.8, 41.2, 22.4 and 20.2% decrease, as compared with control, respectively. FA (200 mg/kg), FH (100 and 200 mg/kg) and FHAppt (100 and 200 mg/kg) showed a significant reduction of the licking activity by 49.5, 57.9, 97.9, 45.3 and 93.7% as compared with control on the second phase, respectively. The inhibitory effect seen with FH and FHAppt at

Table 1. Effects of the Fractions of *Petiveria alliacea* L. on the Abdominal Constriction Induced by Acetic Acid in Mice

Group	Dose (mg/kg)	Number of abdominal constrictions (20 min)	Inhibition (%)
Control	—	36.6±1.70 (46)	—
Indomethacin	2, i.p.	1.0±0.52 (10)[a)]	97.3
FA (pH 6.0)	100, i.p.	3.3±1.01 (19)[a)]	91.1
	200, i.p.	2.7±0.83 (18)[a)]	92.6
FH (pH 5.0)	100, i.p.	2.1±0.64 (20)[a)]	94.2
	200, i.p.	1.7±0.48 (18)[a)]	95.3
FHA (pH 7.0)	100, i.p.	4.9±1.15 (15)[a)]	86.7
	200, i.p.	2.1±0.73 (15)[a)]	94.2
FHAppt (pH 7.0)	100, i.p.	8.3±1.93 (08)[a)]	77.4
	200, i.p.	1.4±0.60 (12)[a)]	96.2

Values are reported as means±S.E.M. for the number of animals shown in parenthesis. *a) vs.* control ($p<0.001$; ANOVA and Student–Newman–Keuls as a *post hoc* test).

Table 2. Effects of the Fractions of *Petiveria alliacea* L. on the Formalin Test in Mice

Group	Dose (mg/kg)	Licking time		Inhibition 1st phase (%)	Inhibition 2nd phase (%)
		1st phase (s)	2nd phase (s)		
Control	—	50.0±1.62 (49)	19.0±1.96 (40)	—	—
Morphine	10, i.p.	5.2±1.81 (06)[a)]	1.8±1.83 (06)[a)]	89.6	90.5
FA	100, i.p.	45.7±3.14 (14)	17.8±3.55 (12)	—	—
	200, i.p.	45.6±3.70 (14)	9.6±2.48 (14)[a)]	—	49.5
FH	100, i.p.	24.3±1.79 (24)[a)]	8.0±2.11 (24)[a)]	51.4	57.9
	200, i.p.	22.3±1.71 (24)[a)]	0.4±0.31 (24)[a)]	55.4	97.9
FHA	100, i.p.	38.8±3.03 (20)[a)]	20.6±2.95 (21)	—	—
	200, i.p.	39.9±2.84 (14)[a)]	15.1±2.94 (14)	20.2	—
FHAppt	100, i.p.	35.6±2.12 (20)[a)]	10.4±1.28 (14)[a)]	28.8	45.3
	200, i.p.	29.4±1.52 (21)[a)]	1.2±0.48 (21)[a)]	41.2	93.7
Nal. +	2, s.c.				
Morphine	10, i.p.	50.0±2.00 (05)[b)]	52.4±4.00 (05)[b)]	—	—
Nal. +	2, s.c.				
FA	200, i.p.	37.0±5.49 (09)	14.5±5.17 (09)	—	—
Nal. +	2, s.c.				
FH	200, i.p.	29.3±3.35 (10)	1.4±1.29 (10)	41.4	92.6
Nal. +	2, s.c.				
FHA	200, i.p.	35.8±4.70 (9)	11.5±3.30 (09)	28.4	—
Nal. +	2, s.c.				
FHAppt	200, i.p.	42.1±1.88 (10)[c)]	3.4±1.39 (10)	—	82.1

Values are reported as means±S.E.M. for the number of animals shown in parenthesis. *a*) *vs*. control; *b*) *vs*. morphine; *c*) *vs*. FHAppt 200 ($p<0.001$; ANOVA and Student–Newman–Keuls as a *post hoc* test).

Table 3. Effects of the Fractions of *Petiveria alliacea* on the Hot-Plate Test in Mice

Group	Dose (mg/kg)	Reaction time (min)			
		0	30	60	90
Control	—	14.9±1.08 (34)	12.6±0.97 (35)	13.4±0.77 (34)	15.4±1.26 (34)
Morphine	10, i.p.	12.3±1.46 (10)	38.6±1.01 (10)[a)]	37.4±1.31 (10)[a)]	38.3±1.36 (09)[a)]
FA	100, i.p.	18.8±1.77 (20)	16.2±1.55 (20)	15.8±1.04 (19)	13.5±1.31 (20)
	200, i.p.	15.5±1.25 (17)	12.5±1.04 (17)	14.0±1.14 (16)	11.5±0.97 (16)
FH	100, i.p.	12.8±1.57 (10)	13.3±1.73 (09)	11.0±1.18 (10)	11.6±1.70 (10)
	200, i.p.	14.1±1.27 (09)	17.4±1.66 (07)	7.1±1.63 (09)	15.0±1.18 (07)
FHA	100, i.p.	15.7±1.49 (18)	17.4±1.38 (16)	18.9±1.81 (18)	15.5±1.39 (19)
	200, i.p.	16.9±1.46 (18)	15.4±1.36 (20)	18.3±1.19 (18)	17.3±1.84 (19)
FHAppt	100, i.p.	18.0±1.20 (17)	19.7±1.04 (17)	17.5±1.30 (17)	14.5±1.09 (17)
	200, i.p.	17.8±1.15 (16)	19.4±1.77 (19)	19.7±1.68 (17)	13.1±1.93 (18)
Naloxone+	2, s.c.				
Morphine	10, i.p.	13.1±1.24 (17)	12.0±0.99 (17)[b)]	12.1±0.64 (18)[b)]	10.6±0.9 2 (17)[b)]

Values are reported as means±S.E.M. for the number of animals shown in parenthesis. *a*) *vs*. control; *b*) *vs*. morphine ($p<0.05$; ANOVA and Student–Newman–Keuls as a *post hoc* test).

second phase was higher than morphine. The only fraction that didn't cause antinociception at first phase on formalin test was FA, and the most effective was FH. The effect seen after the administration of FHAppt 200 mg/kg at first phase were reversed by previous administration of the opioid antagonist, naloxone (2 mg/kg, s.c.).

No significant effect was observed in the hot plate test in mice after treatment with both doses of all the fractions of *Petiveria alliacea* L. as compared to the controls in time zero, whereas morphine (10 mg/kg, i.p.) significantly increased the pain latency (Table 3).

No alteration was observed in the rota rod test after the treatment with both doses of all the fractions of *Petiveria alliacea* L., in contrast, diazepam (2 mg/kg, i.p.) decreased 35.8% the time of permanence on the bar in this test as compared to control (Table 4) showing myorelaxant propertie as expected.

Table 4. Effects of the Fractions of *Petiveria alliacea* L. on the Rota Rod Test in Mice

Group	Dose (mg/kg)	Number of falls	Time of permanence (s)
Control	—	1.87±0.17 (23)	55.0±0.63 (23)
Diazepam	2, i.p.	2.50±0.27 (08)	35.3±6.68 (08)[a)]
FA	100, i.p.	2.20±0.37 (10)	49.7±2.93 (10)
	200, i.p.	1.11±0.35 (10)	55.6±2.55 (10)
FH	100, i.p.	1.80±0.36 (10)	55.6±2.09 (08)
	200, i.p.	1.67±0.41 (09)	56.9±1.72 (07)
FHA	100, i.p.	1.85±0.26 (20)	51.4±2.41 (20)
	200, i.p.	1.16±0.27 (19)	57.3±0.67 (19)
FHAppt	100, i.p.	2.20±0.19 (20)	49.2±3.38 (20)
	200, i.p.	2.11±0.22 (20)	51.4±3.17 (20)

Values are reported as means±S.E.M. for the number of animals shown in parenthesis. *a*) *vs*. control ($p<0.05$; ANOVA and Student–Newman–Keuls as a *post hoc* test).

Table 5. Effects of the Fractions of *Petiveria alliacea* L. on the Open Field Test in Mice

Group	Dose (mg/kg)	Locomotor activity	Inhibition (%)
Control	—	66.0±2.28 (30)	—
Diazepam	2, i.p.	24.3±7.61 (07)*a)*	63.0
FA	100, i.p.	29.2±2.28 (28)*a)*	55.8
	200, i.p.	33.9±1.90 (23)*a)*	48.6
FH	100, i.p.	44.4±3.99 (13)*a)*	32.7
	200, i.p.	36.8±3.17 (17)*a)*	44.2
FHA	100, i.p.	34.3±2.59 (15)*a)*	42.8
	200, i.p.	24.2±2.27 (17)*a)*	63.3
FHAppt	100, i.p.	32.7±2.01 (23)*a)*	50.5
	200, i.p.	31.0±1.89 (29)*a)*	53.0

Values are reported as means±S.E.M. for the number of animals shown in parenthesis. *a*) *vs*. control ($p<0.001$; ANOVA and Student–Newman–Keuls as a *post hoc* test).

Petiveria alliacea L. reduced the locomotor activity in 32.7—63.3% with both doses of all the fractions as compared to control (Table 5). Diazepam (2 mg/kg, i.p.) used as the standard drug caused an inhibition of 63%.

DISCUSSION

The use of different models is significant in the detection of antinociceptive properties in a substance considering that the use of a variety of stimuli recognize different types of pain and reveal the actual nature of antinociceptive test-drug. We investigated the possible antinociceptive effect of the fractions of *Petiveria alliacea* L. on different pain tests.

Past studies have postulated that the acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and opioids.[22] Our results showed inhibitions of abdominal constrictions with all doses of the fractions indicative of antinociceptive effect. Although the abdominal writhes induced by acetic acid represent a peripheral nociception model,[23] this is not a specific model, since several compounds, such as, opioids analgesics,[24,25] tricyclic antidepressants[26] and anti-histaminic[27] inhibit the writhes induced by acetic acid. Even though the tipi prevented the writhes, there is still need for studies using other antinociceptive models.

The formalin test is different from most models of pain in that it is possible to assess the way an animal responds to moderate, continuous pain generated by injured tissue. Because of this connection to tissue injury, it is believed that the test provides a more valid model for clinical pain than the tests with phasic mechanical or thermal stimuli.[28] This model is constituted by two distinct phases. The first phase represents the irritating effects of formalin at the sensorial fibers-C.[29] The second is an inflammatory pain response. This is of interest considering that both phases are sensitive to centrally acting drugs such as opioids,[30] but the second phase is also sensitive to NSAIDs and corticosteroids.[29] Thus, it's possible to appraise the animal's answer to a moderate and continuous pain caused by the tissue lesion as well as the role of pain regulatory endogenous systems. Consequently, through the study of antinociceptive drugs it's possible to evaluate and idenficated the mechanisms of pain and antinociception.

Our results showed that all of the fractions caused significant antinociception on formalin test. FH 200 mg/kg and FHAppt 200 mg/kg demonstrated an antinociceptive action on both phases of this test, and FH was more effective than morphine on the second phase. Naloxone, an opioid receptor prototype antagonist, was able to decrease the antinociceptive action of FHAppt 200 on the first phase. Although this plant presented activity in the first phase of the formalin test, this central protective effect was not corroborated in the hot-plate test and others experiments are necessary to confirm these results.

In this way, to clarify if the analgesic effect of tipi would be consequent to a central activity interference on motor function or motor coordination, we also evaluated the effects of the fractions on open field and rota rod tests that are classical models for screening central nervous system actions providing information about psychomotor performance and myorelaxant activity. The results do not discarded a possible central action of tipi since it decreased locomotor activity, but it not presented myorelaxant activity as demonstrated in the rota rod test, suggesting that the actions observed in this work may not be exerted through peripheral neuromuscular blockade.

These results suggest a possible central mechanism of action mediated mainly, but not exclusively by opioid receptors. Although peripheral mechanisms can't be excluded. This plant decreases the abnormal pain sensitivity, but not normal pain threshold. Furthermore, the different fractions have different antinociceptive potentials.

CONCLUSION

Results showed that the fractions of *Petiveria alliacea* L. possess significant antinociceptive potential, considering the fact that all of the fractions were effective on the abdominal constrictions induced by acetic acid, and formalin tests suggesting peripheral antinociceptive activity. The results of the present work supports the folk medicine use of this plant.

Acknowledgements The authors are grateful to the Brazilian National Research Council (CNPq) for the financial support.

REFERENCES

1) Braga R., "Plantas do nordeste, especialmente do Ceará," 3rd ed., Vol. XLII, Coleção Mossoroense, 1992.
2) Correia M. P., "Dicionário de plantas úteis do Brasil e das exóticas cultivadas," Imprensa Nacional, Vol. I, **3**, Ministerio da Agricultura, Rio de Janeiro, Brazil, 1984, pp. 266—267.
3) Kubec R., Musah R. A., *Phytochemistry*, **58**, 981—985 (2001).
4) Kubec R., Kim S., Musah R. A., *Phytochemistry*, **63**, 37—40 (2003).
5) Coussio J., "270 Plantas Medicinales Iberoaricanas," Cyted, Uneco. Colômbia, 1995.
6) Morales C., Gómez-Serranillos M. P., Iglesias I., Villar A. M., Cáceres A., *Il Fármaco*, **56**, 523—526 (2001).
7) Distasi L. C., Costa M., Medacolli L. J., Kirizana M., Gómes C., Trolin G., *J. Ethnopharmacol*., **24**, 205—211 (1988).
8) De Lima T. C., Morato G. S., Takahashi R. N., *Mem. Inst. O. Cruz*, **86**, 153—158 (1991).
9) De Sousa J. R., Demuner A. J., Pedersoli J. L., Afonso A. M. M., *Ciência e Cultura*, **39**, 646—648 (1987).
10) Lopes-Martins R. A., Pegoraro D. H., Woisky R., Penna S. C., Sertie J. A., *Phytomedicine*, **9**, 245—248 (2002).
11) Rocha A. B., Da Silva J. B., *Rev. Fac. Farm. Odontol*., **3**, 65—72

(1969).
12) Von Szczepanski C., Zgorzelak P., Hoyer G. A., *Arzneim-Forsch*, **22**, 1975—1976 (1972).
13) Adesogan E. K., *J. C. S. Chem. Commun*., **1974**, 906—907 (1974).
14) Benevides P. J. C., Young M. C. M., Giesbrecht A. M., Roque N. F., Bolzani V. S., *Phytochemistry*, **57**, 743—747 (2001)
15) Williams L. A. D., Vasquez E., Klaiber I., Kraus W., Rösner H., *Chemosphere*, **51**, 701—706 (2003).
16) Rösner H., Williams L. A. D., Jung A., Kraus W., *Biochimica et Biophysica Acta*, **1540**, 166—177 (2001).
17) Koster R., Anderson M., De Beer E. J., *Federation Proceeding*, **18**, 412—417 (1959).
18) Hunskaar S., Hole K., *Pain*, **30**, 103—114 (1987).
19) Woolfe G., MacDonald A. D., *J. Pharmacol. Exp. Ther*., **80**, 300—307 (1944).
20) Archer J., *Anim. Behav*., **21**, 205—235 (1973).
21) Dunham N. W., Miya T. S., *J. Am. Pharm. Assoc*., **46**, 208—212 (1957).
22) Collier H. O., Dinneen L. C., Johnson C. A., Schneider C., *Br. J. Pharmacol*., **32**, 295—310 (1968).
23) Wei E. T., Kiang J. G., Buchan P., Smith T. W., *J. Pharmacol. Exp. Ther*., **238**, 783—787 (1986).
24) França D. S., Souza A. L. S., Almeida K. R., Dolabella S. S., Martinelli C., Coellho M. M., *Eur. J. Pharmacol*., **421**, 157—164 (2001).
25) Gonzalez F. G., Portela T. Y., Stipp E. J., Di Stasi L. C., *J. Ethnopharmacol*., **77**, 41—47 (2001).
26) Takahaski R. N., Paz M. M., *Braz. J. Med. Biol. Res*., **20**, 607—610 (1987).
27) Yeh S. Y., *J. Pharmacol. Exp. Ther*., **235**, 683—689 (1985).
28) Tjolsen A., Berge O. G., Hunskaar S., Rosland J. H., Hole K., *Pain*, **51**, 5—17 (1992).
29) Hosseinzadeh H., Ramezani M., Salmani G. A., *J. Ethnopharmacol*, **73**, 379—385 (2000).
30) Shibata M., Ohkubo T., Takahashi H., Inoki R., *Pain*, **38**, 347—352 (1989).