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Full Length Research Paper

Antinociceptive effect of the ethanol crude extract of *Herissantia crispa* (L.) Brizicky

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The species, *Herissantia crispa* (L.) Brizicky, popularly known as malvaísco, is a plant belonging to the Malvaceae family. There are many reports on the pharmacological activity of other species of the Malvaceae family in traditional medicine showing anti-inflammatory, antinociceptive, and diuretic activities. In this study, the antinociceptive effect of the ethanol extract of *H. crispa* (EEHc) was observed in mice. The results showed that EEHc at all doses did not significantly change the latency to thermal stimulus 30, 60 or 120 min after treatment in the hot plate test. The EEHc in the acetic acid-induced writhing test, decreased significantly the number of contortions in the following doses: 250 (1.22 ± 0.57), 500 (2.2 ± 0.87) or 750 mg/kg (3.6 ± 1.42) as compared to the control group (17.78 ± 1.84). EEHc significantly reduced paw licking time at all doses tested in the early and late phase of the formalin test similar to the morphine treated group. The 750 mg/kg dose showed the best effect on phase 1 (54.33 ± 4.95) and phase 2 (13.5 ± 10.68) as compared to the control (95.3 ± 5.32 and 222.8 ± 23.39, subsequently). However, naloxone (NLX) did not reverse the antinociceptive effect of EEHc. Therefore, EEHc showed an antinociceptive activity, but this effect does not involve the opioid system.

Key words: *Herissantia crispa*, Malvaceae, antinociceptive activity, analgesia, natural product.

INTRODUCTION

Plants have been used as medicines by the population, providing good sources of pharmacologically active substances and improving the therapeutic arsenal (Parra et al., 2001). In recent years, there has been a great scientific advancement involving chemical and pharmacological studies of medicinal plants aimed at obtaining new compounds with therapeutic properties (Cechinel-Filho, 2011). Currently, approximately 48% of drugs used in therapy resulted, directly or indirectly, from natural products, especially medicinal plants (Balunas and Kinghorn, 2011). Among many medicinal species of interest, there are plants belonging to the Malvaceae family, and a wide variety of natural compounds found in species of this family possess proven pharmacological properties. When scientifically investigated, the aqueous crude extract of *Sida cordifolia* showed significant anti-inflammatory and antinociceptive effects (Franzotti et al., 2000). The hydroalcoholic crude extract of *S. cordifolia* presented a central nervous system depressant activity (Franco et al., 2005). The crude extract of *Sida rhomboidea* showed excellent antinociceptive and anti-inflammatory effects (Venkatesh et al., 1999). Studies on species of other genus belonging to the Malvaceae family as *Pavonia* and *Abutilon* showed pharmacological activities as emollient, diuretic, and antirheumatic (Ahmed et al., 1990). This family comprises 243 genera and 4225 species, and occurs in almost all parts of the world except very cold regions, being particularly abundant in tropical regions, mainly South America (Heywood, 1993).

Herissantia is a genus of the Malvaceae family which has few studies reported about their chemical and

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pharmacological properties. It consists of six or more species restricted to tropical America (Fryxel, 1997). Several types of phytochemicals, such as fatty acid, triterpenes, steroids, phenolic acid, and flavonoids were isolated from species of this genus (Silva et al., 2005). The plant *Herissantia crispa* (L.) Brizicky, popularly known as malvaísco, belongs to the Malvaceae family (Agra et al., 2008) and there are few pharmacological studies related to this particular species. Pharmacological experiments indicated that the species has antidiarrheal, antiulcer, antimicrobial activity (Lima et al., 2009), and antifungal activity (Johann et al., 2010). Phytochemical study of *H. crispa* resulted in the isolation of seven chemical constituents, three steroids (β-sitosterol, 3-O-β-D-glucopyranoside sitosterol, and stigmasterol 3-O-β-D-glucopyranoside) and four flavonoids: 3,5,7,4 '-tetraidroxiflavonol (kaempferol), 3,5,7,3', 4'-pentaidroxilflavonol (quercetin), kaempferol 3-O-beta-D-glucopyranoside (tiliroside), and kaempferol 3,7-di-O-alpha-L-ramino-piranosídeo (lespedine) (Costa, 2006).

The crude extract of *H. crispa* when evaluated in pharmacological behavioral screening test in our laboratory, presented evidence of depressant activity, and preliminary tests to determine the specific activity, showed a promising analgesic activity. To contribute to the expansion of scientific knowledge about the Malvaceae family, this study aimed to access, the possible antinociceptive effects of the ethanolic crude extract of *H. crispa* in animal models.

MATERIALS AND METHODS

Plant

The aerial parts of *H. crispa* were collected from the Pedra da Boca, Araruna, state of Paraíba, in June 2009, and a voucher specimen (6237) was deposited at the Herbarium of Professor Lauro Pires Xavier (JPB), Federal University of Paraiba.

Extraction

The aerial parts of *H. crispa* (EEHc) were dried in a circulating air oven at 40°C. After that, it was ground in a mechanical mill, and was subjected to maceration in a glass bottle with EtOH:H_2O (95:5) for 72 h. The filtrate was concentrated in a rotary evaporator at 50°C, providing the ethanol extract.

Sample preparation

The crude extract of *H. crispa* was solubilized in distilled water, using when necessary, cremofor (0.2%). All substances were prepared immediately before each experiment, using decimal concentrations in order to enable the administration of 0.1 ml/10 g of body weight. The treatments were performed intraperitoneally (i.p.) where the EEHc was administered at doses of 250, 500, and 750 mg/kg of animal weight. Control groups were treated with a similar volume of vehicle used in the dilution of the experimental group.

Animals

Swiss male mice (25 to 35 g), from the animal house of Prof. Dr. Thomas George, Federal University of Paraíba (UFPB), Brazil, were used. The animals were housed in cages with free access to food and water. All animals were kept under 12 h light/dark cycle (lights on at 6:00 a.m). The animals were treated according to the ethical principles of animal experimentation of Brazilian College of Animal Experiments (COBEA), Brazil, and the rules of the National Institute of Health Guide for Care and Use of Laboratory Animals. The animal studies Committee of the Federal University of Paraíba, approved the experimental protocols (number 0607/08).

Hot-plate test

The animals were placed on the hot plate (51 ± 1°C), 30 min after treatment, and the latency time was recorded, which is the time between placing the animal in the preheated plate and the attempt by the animal to jump or lick a paw. This procedure was repeated 60 and 120 min after administration. The animals remained on board for a maximum time of 30 s to prevent tissue damage. Morphine (10 mg/kg i.p.) was used as standard drug and positive control (Almeida and Oliveira, 2006).

Acetic acid-induced writhing test

The animals were divided into five groups and treated with vehicle, EEHc (250, 500 or 750 mg/kg), and morphine (6 mg/kg). Thirty minutes after, the animals were injected with a solution of 1% acetic acid in distilled water (0.1 ml/10 g) and were placed in individual polyethylene cages. Five minutes later, the number of writhing of each animal was registered during 10 min. A significant reduction in the number of contortions when compared with the control group is considered an antinociceptive response (Bastos et al., 2006).

Formalin test

The animals were divided into five groups and treated with vehicle, EEHc (250, 500 or 750mg/kg), and morphine (10 mg/kg). After 30 min, 20 µl of a 2.5% formalin solution was injected into the subplantar region of the right hind paw. The duration of paw licking was measured at 0 to 5 min (first phase) and 15 to 30 min (second phase) after formalin administration (Vaz et al., 1996).

Participation of the opioid system

To investigate the possible participation of the opioid system on the antinociceptive effect of EEHc, the animals were pre-treated with naloxone (NLX, a non-selective opioid antagonist) at a dose of 6 mg/kg (s.c.); 15 min after, the test group received treatment with EEHc (750 mg/kg) and the standard group received morphine (10 mg/kg). The other group (control) received only vehicle, and after 30 min, all groups were tested with formalin (Tjolse et al., 1992). The parameter measured was the duration of paw licking.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) and statistically analyzed by one way analysis of variance (ANOVA), followed by Dunnett's test for parametric measures and Kruskal-Wallis test, followed by Dunn's multiple comparison for nonparametric measures. The results were considered statistically significant when $P < 0.05$. All data were analyzed using GraphPad

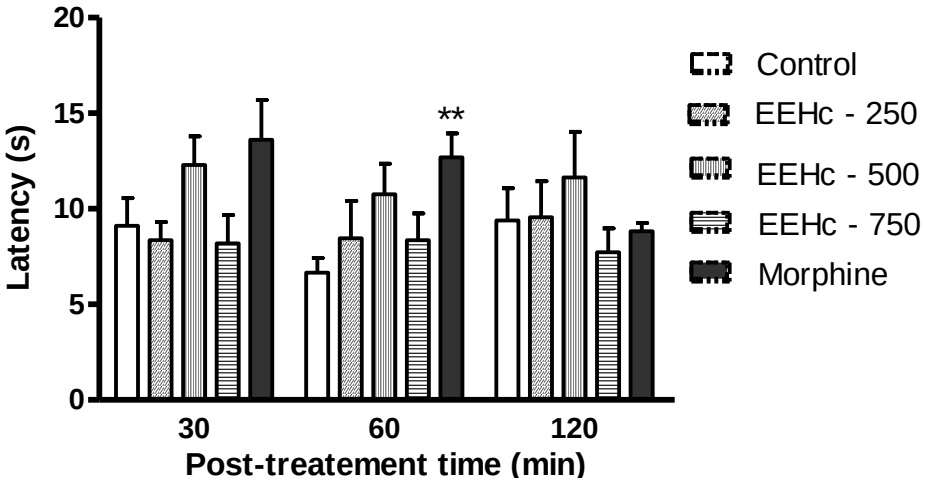

Figure 1. Effect of EEHc and morphine (10 mg/kg) in mice on the latency time in the hot plate test, 30, 60, and 120 min after treatment of animals. Values are expressed as mean ± SEM (n = 10). **P < 0.01 (one-way ANOVA followed by Dunnett's post test).

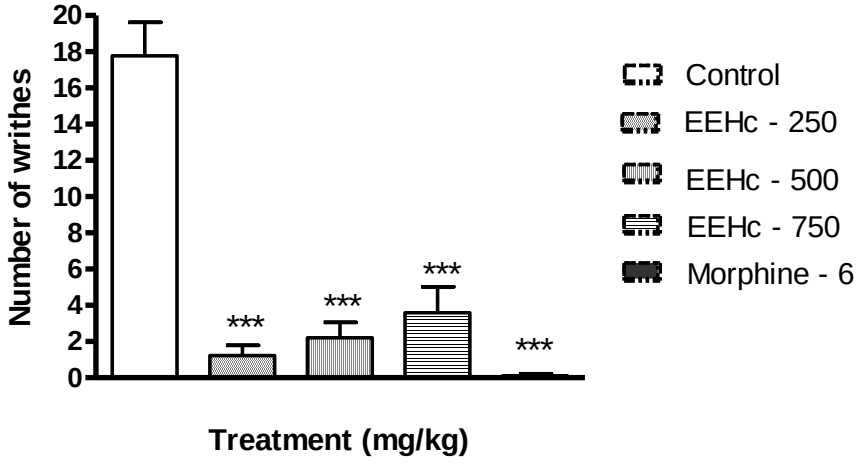

Figure 2. Effect of EEHc and morphine in mice, in the number of abdominal contortions induced by acetic acid. Values are expressed as mean ± SEM (n = 10). ***P < 0.001 (one-way ANOVA followed by Dunnett's post test).

Prism, version 5.0 (GraphPad Software Incorporated, San Diego, USA).

RESULTS

Hot plate test

There was no significant variation in latency to thermal stimulus 30, 60 or 120 min after administration of EEHc (250, 500, and 750 mg/kg, i.p.) as compared to the control group (Figure 1). Morphine (10 mg/kg, i.p.) used as standard substance in this test, showed a significant increase in latency time, 60 min after administration. However, morphine did not change latency time significantly, 30 and 120 min as compared to the control.

Acetic acid-induced writhing test

The EEHc significantly reduced the number of writhings caused by acetic acid administration, at all three doses tested: 1.22 ± 0.57 at the dose of 250 mg/kg, 2.2 ± 0.87 at the dose of 500 mg/kg, and 3.6 ± 1.42 at the dose of 750 mg/kg, i.p., as compared to control (17.78 ± 1.84). Similarly, morphine (6 mg/kg, i.p.) also caused a reduction in the number of writings in animals (Figure 2). The results did not differ statistically among treatment groups.

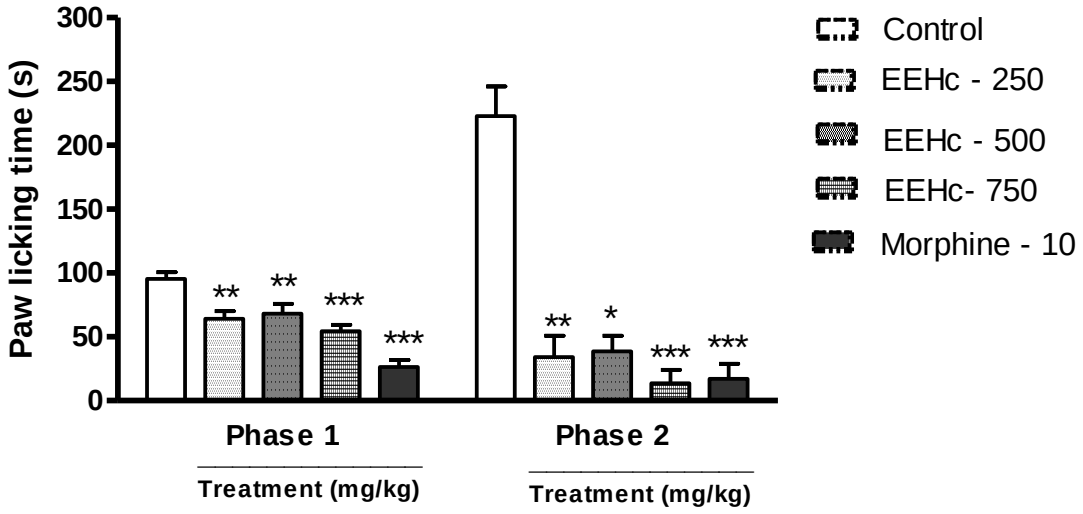

Figure 3. Effect of EEHc and morphine in mice in the first and second phase of the formalin test. Values are expressed as mean ± SEM (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001 (one-way ANOVA followed by Dunnett's post test (phase 1) or Kruskal-Wallis's followed by Dunn's post-test (phase 2)).

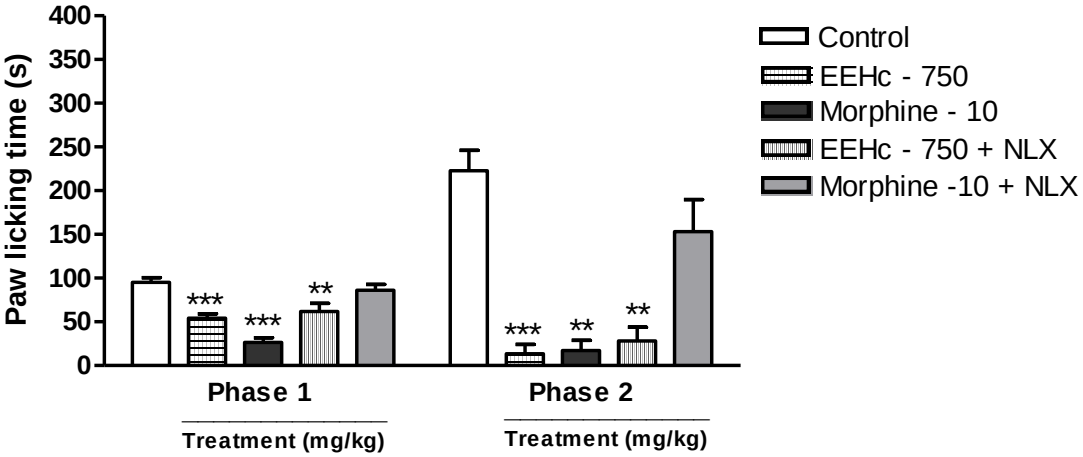

Figure 4. Effect EEHc and morphine in mice in the first phase and second phase of the formalin test after pre-treatment with naloxone (NLX, 6 mg/kg). Values are expressed as mean ± SEM (n = 10). **P < 0.01, ***P < 0.001 (one-way ANOVA followed by Dunnett´s post test (phase 1) or Kruskal-Wallis's followed by Dunn's post-test (phase 2)).

Formalin test

In the early and late phase of the test, mice treated with EEHc (250, 500, and 750 mg/kg, i.p.) showed a significant decrease in paw licking time as compared to the control. The inhibitions observed were 64.11 ± 5.99, 68.22 ± 7.62, and 54.33 ± 4.95% at doses of 250, 500, and 750 mg/kg respectively, as compared to the control (95.30 ± 5.32) in the first phase and 34.20 ± 16.77, 38.56 ± 12.32, and 13.50 ±10.68 mg/kg, respectively as compared to control (222.8 ± 23.39) in the second phase. The standard group treated with morphine (10 mg/kg) inhibited 26.40 ± 5.58 in the first phase and 17.0 ± 11.76 in the second phase (Figure 3).

Participation of opioid system

The pre-treatment of mice with NLX did not affect the antinociceptive effect of EEHc (750 mg/kg) in paw licking time in early and late phases of the formalin test, but reversed the antinociception caused by morphine (10 mg/kg), since the drug acts via opioid receptors (Figure 4). The inhibition values were: 54.33 ± 4.95 (750 mg/kg), 62.20 ± 9.33 (750 + NLX), 28.33 ± 6.1 (morphine), and 86.10 ± 6.64 (morphine + NLX) to phase 1 and 13.5 ±

10.68 (750 mg/kg), 28.11 ± 15.85 (750 + NLX), 17.9 ± 10.89 (morphine), and 153.2 ± 36.78 (morphine + NLX) to phase 2.

DISCUSSION

The investigation of medicinal plants from the information provided by folk medicine and herbal medicine has gained support in scientific research, aiming to develop new effective drugs that do not exhibit toxic effect or have low toxicity (Taylor et al., 2001). Although, *H. crispa* species does not have ethnopharmacological indication, it was selected for study because of some isolated flavonoids with proven activity (Dimas et al., 2000; Matsuda et al., 2002; Jorge, 2003; Sala et al., 2003; Silva et al., 2005; Costa, 2006; Costa et al., 2007). In this study, we assessed the possible antinociceptive effects of *H. crispa* (EEHc) using nociceptive models. The EEHc showed no effect in the hot plate test, which is a test used to evaluate the analgesic activity mediated by central mechanisms (Antoniolli and Villar, 2003). However, treatment of mice with morphine resulted in significant increase in the latency to the thermal stimulus. In the acetic acid-induced writhing test, which is a highly sensitive method to test centrally and/or peripheral acting drugs (Vaz et al., 1996), EEHc reduced the number of writhings in all the three doses tested. This indicates that the EEHc has antinociceptive activity and/or inhibits the release of inflammatory mediators or cytokines. The nociceptive response to acetic acid may involve a direct stimulation of nociceptive afferent fibers, due to a pH decrease or a synthesis of inflammatory mediators, such as metabolites of arachidonic acid through cyclo-oxygenase, with subsequent biosynthesis of prosta-glandins (Franzotti et al., 2000). Formalin produced a distinct biphasic response where analgesic drugs may act differently in early and late phases of the test (Morteza-Semnan et al., 2002). The early phase results from direct chemical stimulation of nociceptive afferents, especially C fibers and release of substance P, and can be inhibited by centrally acting drugs such as morphine (Heapy et al., 1987). The late phase results from the action of inflammatory mediators released locally, such as prostaglandins, serotonin, histamine, and bradykinin (Murray et al., 1988), or by facilitation of spinal synaptic transmission (Tjolsen et al., 1992). Drugs acting centrally, inhibit both phases of the formalin test; however, peripheral acting drugs are effective only in the late phase (Shibata et al., 1989). Like other substances that act on the central nervous system, the EEHc inhibited both phases of the test similar to morphine. Therefore, these results confirm the central antinociceptive effect of *H. crispa.*

Among the neurotransmitter systems involved in pain, the opioid system is one of the most important (Hess et al., 2010). Our results showed that the antinociceptive effect of EEHc does not involve the participation of the opioid system since NLX, an opioid antagonist, reversed the antinociception caused by morphine without affecting antinociception produced by EEHc, in the formalin test.

Conclusively, this study demonstrated that the EEHc have antinociceptive activity in the central nervous system. However, the EEHc does not exert its effects through the activation of receptors opioids. Additional studies are needed to investigate the mechanism of action of EEHc in the mechanisms of pain inhibition in the central nervous system.

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REFERENCES

Agra MF, Silva KN, Basílio IJLD, Freitas PF, Barbosa-Filho JM (2008). Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn. 18 (3):472-508.

Ahmed Z, Kazmi SNH, Malik A (1990). A New Pentacyclic Triterpene from *Abutilon pakistanicum*. J. Nat. Prod. 53(5):1342-1344

Almeida FRC, Oliveira FS (2006). Avaliação de drogas analgésicas de ação central. In: Almeida, RN Psicofarmacologia: fundamentos práticos, 1ª ed. Rio de Janeiro, Guanabara Koogan, pp. 179-188.

Almeida CF, Amorim EL, Albuquerque UP (2011). Insights into the search for new drugs from traditional knowledge: an ethnobotanical and chemical-ecological perspective. Pharm Biol. 49:864-873.

Antoniolli AR, Villar JC (2003). Atividade antinociceptiva e toxicidade aguda do extrato aquoso de *Vitex agnus-castus* L. Anais do seminário de Pesquisa FAP-SE.

Balunas MJ, Kinghorn D (2005) Drug discovery from medicinal plants. Life Sci. 78:431-41.

Bastos GNT, Santos ARS, Ferreira VMM, Costa AMR, Bispo CI, Silveira AJA, Nascimento JLM (2006). Antinociceptive effect of the aqueous extract obtained from roots of *Physalis angulata* L. in mice. J. Ethnopharmacol. 103:241-245.

Cechinel-Filho V (2011). A Rede RIBIOFAR/CYTED/CNPq e suas implicações na busca de princípios ativos de origem natural. Revista Fitos (ALANAC) 6:57-64.

Costa DA (2006) Constituintes químicos de *Bakeridesia pickelii* (H Monteiro) e *Herissantia crispa* L. (Brizicky) (Malvaceae). PhD thesis, Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Brasil.

Costa AD, Silva DA, Cavalcanti AC, Medeiros MAA, Lima JT, Cavalcante JMS, Silva BA, Agra MF, Souza MFV (2007). Chemical constituents from *Bakeridesia pickelii* Monteiro (Malvaceae) and the relaxant activity of kaempferol-3-o-β-d-(6”-e-p-coumaroyl) glucopyranoside on guinea-pig ileum. Quim. Nova 30(4):901-903.

Dimas K, Demetzos C, Mitaku S, Marselos M, Tzavaras T, Kokkinopoulos D (2000). Citotoxic activity of kaempferol glycosides against human leukaemic cells lines *in vitro*. Pharmacol. Res. 41:85-88.

Franzotti EM, Santos CVF, Rodrigues HMSL, Mourão RHV, Andrade MR, Antoniolli AR (2000). Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). J. Ethnopharmacol. 72:273-278.

Fryxell PA (1997). The American genera of Malvaceae. II. Britonia, 49:204-269.

Heapy CG, Jamieson A, Russel NJW (1987). Afferent C-fibre and A-δ activity in models of inflammation. Br. J. Pharmacol. 90:164.

Hess S, Padoani C, Scorteganha LC, Holzmann I, Malheiros A, Yunes

RA, Monache FD, Souza MM (2010). Assessment of Mechanisms Involved in Antinociception Caused by Myrsinoic Acid B. Biol. Pharm. Bull. 33(2):209-215.
Heywood VH (1993). Flowering Plants on the World, Ed. B. T. Batsford Ltda, London.
Jorge AP (2003). Efeito insulino-mimético do canferol 3,7-O-(α)-L-diraminosídeo na glicemia e na captação da 2-[14C (U)]-deoxi-D-glicose em músculo sóleo de ratos. MSc. dissertation, Universidade Federal de Santa Catarina, Brasil.
Johann S, Cisalpino PS, Watanabe GA, Cota BB, Siqueira EP, Pizzolatti MG, Zani CL, Resende MA (2010). Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*. Pharm Biol. 48:388-396.
Lima IO, Costa VBM, Matias WN, Costa DA, Silva DA, Agra MF, Souza MFV, Lima EO, Batista LM (2009). Biological activity of *Herissantia crispa (L.)* Brizicky. Rev. Bras. Farmacogn. 19:249-254.
Matsuda H, Ninomiya K, Shimoda H, Yoshikawa M (2002). Hepatoprotective principles from the flowers of *Tilia argentea* (Linden): Structure requirements of tiliroside and mechanisms of action. Bioorg. Med. Chem. 10:707-712.
Morteza-Semnani K, Saeedi M, Hamidian M, Vafamehr H, Dehpour AR (2002). Anti-inflammatory, analgesic activity and acute toxicity of *Glaucium grandiflorum* extract. J. Ethnopharmacol. 80:181-186.
Murray CW, Porreca F, Cowan A (1988). Methodological refinements in the mouse paw formalin test an animal model of tonic pain. J. Pharmacol. Meth. 20:175-186.
Parra AL, Yhebra RS, Sardiñas IG, Buela LI (2001). Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine 8:395-400.
Sala A, Recio MC, Schinella GR, Manez S, Giner RM, Cerdá- Nicolás M, Ríos JL (2003). Assement of the radical scanvenger activity of tiliroside. Eur. J. Pharmacol. 461:53-61.
Shibata M, Ohkubo T, Takahashi H, Inoki R (1989). Modified formalin test: characteristic biphasic pain response. Pain 38:347-352.
Silva DA, Costa DA, Silva DF, Souza MFV, Agra MF, Medeiros IA, Barbosa-Filho, JM, Braz-Filho, R (2005). Flavonóides glicosilados de *Herissantia tiubae* (K. Schum) Brizicky (Malvaceae) e testes farmacológicos preliminares do canferol 3,7-di-O-α-L-ramnopiranosídeo. Rev. Bras. Farmacogn. 15(1):23-29.
Taylor JLS, Rabe T, McGaw LJ, Jäger AK, Van Staden, J (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 34:23-37.
Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K (1992). The formalin test: an evaluation of the method. Pain 51:5-17.
Vaz ZR, Cechinel-Filho V, Yunes RA, Calixto JB (1996). Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxyline derivative on chemical and thermal models of nociceptive in mice. J. Pharmacol. Exp. Ther. 278:304-312.
Venkatesh S, Reddy SR, Suresh B, Ressy BM, Ramesh M (1999). Antinociceptive and Anti-inflammatory Activity of *Sida rhomboidea* Leaves. J. Ethnopharmacol. 67:229-232.