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Artigo

Analgesic and anti-inflammatory effects of *Cheiloclinium cognatum* root barks

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RESUMO: "Efeitos analgésico e antiinflamatório das cascas das raízes de *Cheiloclinium cognatum*". *Cheiloclinium cognatum* é uma planta da família das Hippocrateaceae, popularmente conhecida como bacupari, usada popularmente no tratamento de febre e edemas. Neste artigo, descrevemos as atividades antiinflamatória e analgésica do extrato bruto diclorometânico das cascas das raízes (DECc), coletadas no bosque Auguste de Saint Hilaire, localizado na Universidade Federal de Goiás. Doses de 0,1, 0,3 e 1,0 g/kg causaram uma redução de 21, 30 e 51%, respectivamente, no edema de orelha de camundongos, induzido pelo óleo de cróton. No método algesimétrico, flexão de cauda, observou-se um significativo aumento no tempo para reação ao estímulo térmico equivalente a 105, 189 e 200%. Estes resultados sugerem que *C. cognatum* pode ser uma fonte de novos compostos com atividades antiinflamatória e analgésica.

Unitermos: *Cheiloclinium cognatum*, Hippocrateaceae, efeito antiinflamatório, atividade analgésica, edema de orelha, peritonite.

ABSTRACT: *Cheiloclinium cognatum* (Hippocrateaceae) has been used in folk medicine to treat fever and edema. In this paper, we report the anti-inflammatory and analgesic activities of the crude dichloromethane extract (DECc) from *C. cognatum* root barks collected in Auguste de Saint Hilaire wood at Universidade Federal de Goiás. Doses of 0.1, 0.3 and 1.0 g/kg caused a dose-dependent inhibition of croton oil-induced ear edema in mice equivalent to 21, 30 and 51%, respectively. There was a significant increase in analgesic-meter-induced tail flick test equivalent to 105, 189 and 200% of increase tail flick reaction time. These results allowed to suggest that *C. cognatum* could be a source of new compounds which anti-inflammatory and analgesic activities.

Keywords: *Cheiloclinium cognatum*, Hippocrateaceae, anti-inflammatory effect, analgesic activity, ear edema, peritonitis.

INTRODUCTION

Plants of Celastraceae and Hippocrateaceae families, particularly in central Brazilian cerrado region, have been classified in the category of a threatened Brazilian species (Pinto, 1994), due to its population decline. The latter is a consequence of its excessive exploitation and destruction of its habitats, as well as its low seed viability, which makes it difficult to propagate. In the course of our systematic studies on the Brazilian Cerrado plants, especially of these families (Lião et al., 2001, 2002), we have investigated *Cheiloclinium cognatum* (Miers) A. C. Smith (Hippocrateaceae), a tree widely distributed in Brazilian cerrado region, popularly known as "bacupari". This plant has been used in folk medicine to treat fever and edema. The Celastraceae and Hippocrateaceae families are rich sources of quinonemethide triterpenes (Gunatilaka, 1996), and these compounds have a variety of biological activities such as antitumor (Gunatilaka, 1996; De Lima et al., 1971), antimicrobial (Gunatilaka, 1996; De Santana et al., 1971), antibiotic (De Lima et al., 1969), cytotoxic (Kutney et al., 1981; Setzer, 1998), antimalarial (Pavanand et al., 1989; Figueiredo, 1998), trypanocidal (Goijman et al., 1985), and anti-inflammatory (Allison et al., 2001). In this paper, we report the anti-inflammatory and analgesic activity of the crude dichloromethane extract from *C. cognatum* root barks.

MATERIAL AND METHODS

Plant material

The root barks of *Cheiloclinium cognatum* (Hippocrateaceae) were collected in Auguste de Saint Hilaire wood at Universidade Federal de Goiás, Goiânia,

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Goiás State, Brazil (S 16º36'11"; W 49º15'39"; 695m), in April 2000, and identified by Dr Júlio Antônio Lombardi (Departamento de Botânica do Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, UFMG). The voucher specimen (19797) is deposited at Herbarium of Instituto de Ciências Biológicas (UFG), Goiás.

Preparation of extract

The root barks of *C. cognatum* were dried in a circulating air stove and grounded. The powder was extracted with CH_2Cl_2 by maceration for 3 weeks at room temperature. Solvent was removed by distillation under reduced pressure affording dichloromethane extract (DECc - 3.0 g).

Animals

Swiss albino male mice (90 days of age), weighting 28-32 g were obtained from the Central Animal House of the Universidade Federal de Goiás (UFG). The animals were maintained at 22 ± 2 °C on a 12/12 h light-dark cycle (light from 07:00 am to 07:00 pm), with food and water available *ad libitum*, in accordance to The Guide for the Care and Use of Laboratory Animal, National Research Council, USA (1996). The experimental protocols used in this study were approved by the Institutional Ethics Committee (CEPMHA/HC/UFG Nº 005/06).

Drugs

Croton oil, heparin and dexamethasone were obtained from Prodome (Brazil); morphine was obtained from Roche (Switzerland); sodium pentobarbital from Abbott (Brazil); and indomethacin, carrageenan and other used chemicals were purchased from Merck (Brazil).

Effect on gross behavior

The behavioural screening of the mice was performed following parameters described by Malone (1977). The DECc (doses from 0.01 to 1.0 g/kg) or vehicle (tween 80/saline - 0.01 mL/mL, 10 mL/kg) was administered to four groups of five mice each by *p.o.*, s.c. or i.p. The animals were kept under observation for seven days after drug administration.

Pentobarbital-induced sleep

Animals were divided in 4 groups of 11 mice each. Sixty minutes after oral administration of DECc (0.1, 0.3, or 1.0 g/kg) or vehicle (10 mL/kg), all mice were injected with sodium pentobarbital (50 mg/kg, i.p.). The time between loss and subsequent recovery of the righting reflex was taken as the sleeping time and was recorded for animals pre-treated with vehicle or drug (Carlini; Burgos, 1979). The results were expressed as relative percentages to the control group.

Acetic acid-induced abdominal writhing

The response to i.p. injection of an acetic acid solution is a contraction of the abdominal muscle and stretching of the hind limbs, induced according to procedures described by Hendershot and Forsaith (1959). Animals were divided in 5 groups of 8 mice each and treated orally with indomethacin (10 mg/kg, *p.o.*), used as positive control, or DECc (0.1, 0.3, or 1.0 g/kg, *p.o.*) 60 min before the administration of 1.2% (v/v) acetic acid (10 mL/kg; i.p.). The number of abdominal writhings produced in each group for the subsequent 30 min was counted and the results were expressed as percentages in relation to the control group treated with vehicle (control, 10 mL/kg).

Tail flick test

The reaction of mice to thermal stimulation of the tail tip by immersion in water maintained at 55.5 ± 0.5 °C was recorded at 30 min intervals before, and 0, 30, 60, 90, and 120 min after treatment. The mice were divided into five experimental groups (n = 7, each one) consisting of animals treated with DECc (0.1, 0.3, 1.0 g/kg *p.o.*), vehicle (10 mL/kg, *p.o.*) or morphine (5 mg/kg, s.c.) used as positive control. The analgesia data are expressed as mean ± SEM, as percentages relative to time 0, according to Janssen et al. (1963), as adapted by Grotto and Sulman (1967).

Croton oil-induced ear edema test

Animals were divided in 5 groups of 7 mice each and treated with vehicle (control, 10 ml/kg; *p.o.*), or dexamethasone (2 mg/kg; *p.o.*), used as positive control, or DECc (0.1, 0.3, 1.0 g/kg *p.o.*), and 60 minutes later, cutaneous inflammation was induced by applying 25 µL of croton oil in acetone (2.5% v/v) to the inner surface of the right ear. The same volume of acetone was applied to the left ear by the method of Zanini et al. (1992). Four hours after treatment, mice were killed by cervical dislocation and a 6 mm diameter punch biopsy was taken from both the treated and untreated ears. The inflammatory response (edema) was monitored by measuring the differences in weight (mg) between the two plugs.

Statistical analysis

The results were analyzed by one-way ANOVA followed by Student's t-test for unpaired samples (Sokal; Rohlf, 1981). The data were expressed as mean ± SEM.

P values less than 0.05 ($p < 0.05$) were considered as indicative of significance.

RESULTS AND DISCUSSION

In the general pharmacological activity test, the DECc elicited a rapid-onset dose-related decrease in spontaneous motor activity and analgesia. After i.p. administration (0.1, 0.3, and 1.0 g/kg) the effects were more intensive than after subcutaneous or oral administration. When administered by via i.p. the DECc (1.0 g/kg) caused death 30 min and subcutaneous 24 h after treatment. No deaths occurred in the control group. Neither effects such as the appearance of abdominal contortion, palpebral ptosis, piloerection, aggression, tremors or convulsions were observed. The decrease of the spontaneous motor activity observed in the general pharmacological activities test suggested the presence of compounds with central depressive action. This action of DECc was confirmed with the results obtained in the duration sleeping time by sodium pentobarbital in mice previously treated with different doses of DECc (Figure 1). In the sleep test induced by sodium pentobarbital the animals treated with DECc at 0.3 and 1.0 g/kg doses (*p.o.*) presented increase of sleep time in 21 and 63% respectively, when compared to control (72.4 ± 5.2 min). This result confirms the presence of compounds with central depressive action in DECc, according observed in the general pharmacological activities test.

In evaluation of analgesic activity reduction of 29.3 and 49.3% in the number of writhings in the animals groups treated with DECc extract at 0.3 and 1.0 g/kg *p.o.* doses, was observed. This result was significant when compared with control which showed 71 ± 3.4% (Figure 2). The reduction in the number of writhings in the groups of treated mice was considered to be a positive analgesic response. Although this test has been a non-specific model it is widely used for analgesic screening and involves cholinergic and histaminic peritoneal receptors and the mediator's acetylcholine and histamine (Fujiyoshi et al., 1989). Collier et al. (1968) proposed that acetic acid acts indirectly by releasing endogenous mediators that stimulate the nociceptive effect but also stimulates neurons that are sensitive to other drugs such as narcotics and centrally acting agents.

In the tail flick test, the time required for mouse tail flick after exposure to source of thermal was significantly increased after 30 minutes of the treatment with DECc extract in 0.3 and 1.0 g/kg *p.o.* doses (Figure 3). It is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat. The significant increase in the pain threshold caused by the DECc extract in the tail flick model could contemplate a non-specific depressant action or reduction of motor coordination of animals (Kim et al., 2004).

It was also observed that the DECc extract in 0.1, 0.3 and 1.0 g/kg *p.o.* doses significantly inhibited the croton oil-induced ear edema response in a dose-dependent manner (Figure 4). The decrease was 21, 30 and 51% respectively, compared with control (21 ± 1.4 mg).

In order to distinguish between the central analgesic action and peripheral anti-inflammatory activity, response croton oil-induced ear edema test in mice was used. This experimental model is sensitive for steroidal and non-steroidal anti-inflammatory compounds (Puigneró et al., 1998; Falcão et al., 2005; Medeiros et al., 2007), behaving us to conclude that DECc contains compounds with depressive central and/or analgesic activities, besides to present anti-inflammatory effect. Using isolated substances we could elucidate the involved action mechanism.

Association of results obtained by abdominal writhing movements and tail flick reaction time suggested that the analgesic activity could be caused by a depressive action in central nervous system, confirmed by increase in pentobarbital-induced sleep time. Otherwise the reduction observed in croton oil-induced ear edema test showed an anti-inflammatory activity. This action could be reducing the abdominal writhing movements in similar form as in aspirin like (AINS), reducing the prostaglandins synthesis by inhibition of cycloxygenase-1 and -2 (COX-1 and COX-2) (Vane, 1971; Vane; Booting, 1996), the enzymes that convert arachidonic acid to prostaglandins (Xie et al., 1992) and are involved in modulating fever (Milton; Wendlandt, 1970) and inflammatory pain (Ferreira, 1972, 1990).

Previous work on the ethanol extract of *Tripterygium wilfordii*, a plant of Celastraceae family, showed the presence of the quinonemethide triterpenoid celastrol as a suppressor of adjuvant arthritis in the rat, demonstrating *in vivo* anti-inflammatory activity (Allison et al., 2001). Quinonemethide triterpenoids constitute a relatively small group of natural products encountered exclusively in plants of Celastraceae and Hippocrateaceae.

Recent phytochemical studies of *C. cognatum* extracts have been shown the presence of the quinonemethide triterpenoids pristimerin, maytenin, 22β-hydroxymaytenin, 20α-hydroxymaytenin, netzahualcoyonol, netzahualcoyondiol and netzahualcoyone (Jeller et al., 2004), suggesting that the anti-inflammatory response could be associated to these compounds. Further studies will be performed to corroborate this association.

In summary the anti-inflammatory and analgesic effects induced by the crude dichloromethan extract from *C. cognatum* root barks support the popular use of the "bacupari" in fever and edema treatment.

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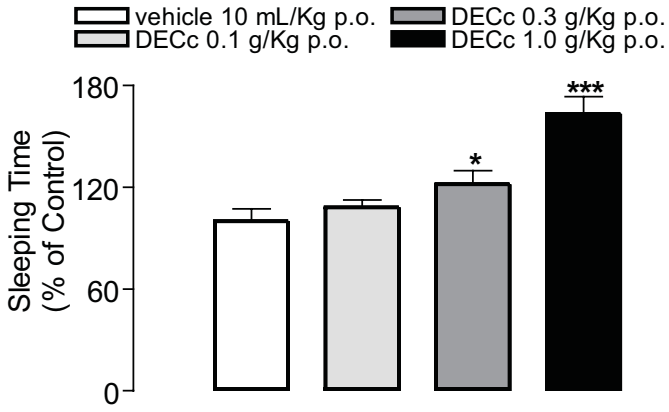

Figure 1. Effect of the crude dichloromethan extract from *Cheiloclinium cognatum* root barks (0.1, 0.3 or 1.0 g/kg, *p.o.*) on the sleeping time induced by sodium pentobarbital (50 mg/kg, i.p.) in mice. The vertical bars indicate the means ± SEM, expressed as relative percentage to the control group. * $p < 0.05$, *** $p < 0.001$.

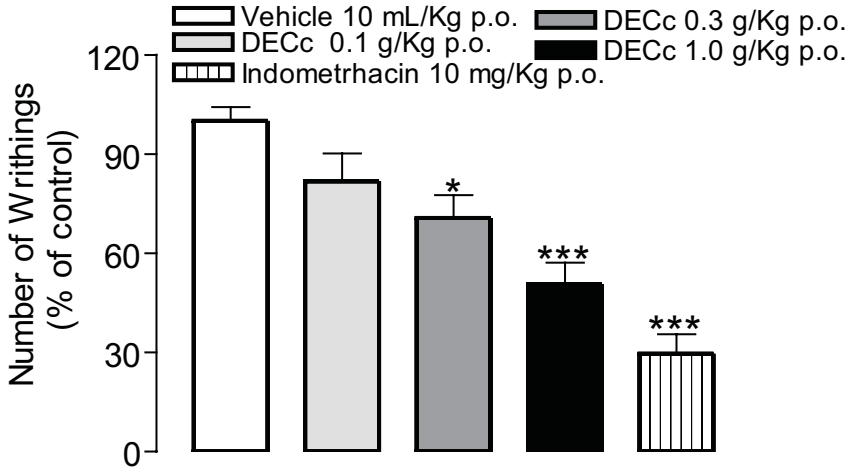

Figure 2. Effect of the crude dichloromethan extract from *Cheiloclinium cognatum* root barks (0.1, 0.3 or 1.0 g/kg, *p.o.*) on the number of acetic acid-induced abdominal writhing movements. The vertical bars indicate the means ± SEM, expressed as relative percentage to the control group. Indomethacin (10 mg/kg, *p.o.*) was used as a positive control. * $p < 0.05$, *** $p < 0.001$.

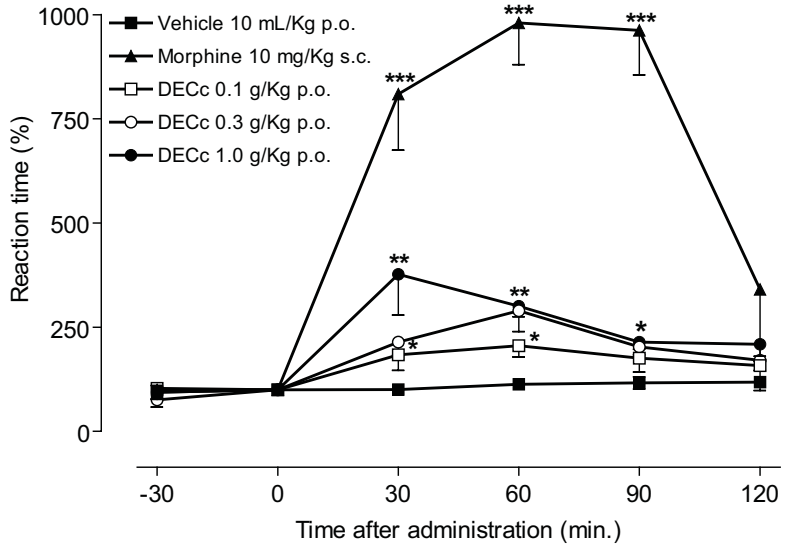

Figure 3. Effect of pre-treatment with crude dichloromethan extract from *Cheiloclinium cognatum* root barks (0.1, 0.3 or 1.0 g/kg, *p.o.*) in the tail flick test. Morphine was used as a positive control. The points indicate the means ± SEM, expressed as relative percentage to the zero time. * $p < 0.05$, ** $p < 0.0l$ and *** $p < 0.001$.

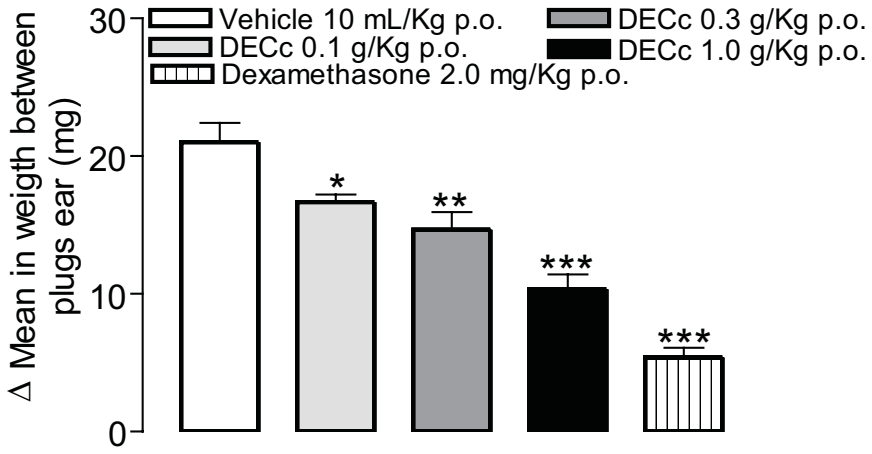

Figure 4. Effect of the crude dichloromethan extract from *Cheiloclinium cognatum* root barks (0.1, 0.3 or 1.0 g/kg, *p.o.*) on croton oil-induced ear edema in mice. The vertical bars indicate the means ± SEM of differences in weight between right and left plugs ear. Dexamethasone (2.0 mg/kg, *p.o.*) was used as a positive control. *p < 0.05, **p < 0.01 and *** p < 0.001.

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REFERENCES

Allison AC, Cacabelos R, Lombardi VRM, Alvarez XA, Vigo C 2001. Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. *Prog Neuro-Psychoph 25*: 1341-1357.

Carlini EA, Burgos V 1979. Screening farmacológico de ansiolíticos: Metodologia laboratorial e comparação entre o diazepam e o clorobenzepam. *Rev Assoc Bras Psiquiatria 1*: 25-31.

Collier HOJ, Dinneen LC, Johnson CA, Schneider C 1968. The abdominal constriction response and its suppression by analgesics drugs in the mouse. *Brit J Pharmacol 32*: 295-310.

De Lima OG, D'Albuquerque IL, Maciel GM 1969. Substâncias antimicrobianas de plantas superiores. Comunicação XXIX. Primeiras observações sobre a atividade antimicrobiana de celastrol. *Rev Inst Antibióticos 9*: 75-77.

De Lima OG, D'Albuquerque IL, De Barros JS, Coelho DG, Martins AL, Lacerda GM 1971. Substâncias antimicrobianas de plantas superiores. Comunicação XXXVI. Sobre a presença de maitenina e pristimerina na parte cortical das raízes de *Maytenus ilicifolia*, procedente do Brasil Meridional. *Rev Inst Antibióticos 11*: 35-38.

De Santana FC, Cortias CT, Pinto KV, Satiro MW, Satiro AL, Lacerda AL, Moreira IC 1971. Primeiras observações sobre o emprego de maitenina em pacientes cancerosos. *Rev Inst Antibióticos 11*: 37-49.

Falcão HS, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, Batista LM 2005. Review of the plants with anti-inflammatory activity studied in Brazil. *Rev Bras Farmacogn 15*: 381-391.

Ferreira SH 1972. Prostaglandins, aspirin-like drugs and analgesia. *Nature-New Biol 240*: 200-203.

Ferreira SH, 1990. A classification of peripheral analgesics based upon their mode of action. In: Sandler, M., Collin, G. (Eds.), Migraine a Spectrum of Ideas. Oxford Medical Publications, New York.

Figueiredo JN, Raz B, Sequin U 1998. Novel quinonemethides from *Salacia kraussii* with *in vitro* antimalarial activity. *J Nat Prod 61*: 718-723.

Fujiyoshi T, Hayashi I, Ohishi S, Kuwashima M, Iida H, Dozen M, Taniguchi N, Ikeda K, Ohnishi H 1989. Kaolin-induced writhing in mice, a new model of possible bradykinin-induced pain for assessment of analgesic agents. *Agent and Actions 27*: 332-334.

Goijman SG, Turrens JF, Marini-Bettolo GB, Stoppani AOM 1985. Effect of tingenone, a quinonoid triterpenes, on growth and macromolecule biosynthesis in *Trypanosoma cruzi*. *Experientia 41*: 646-648.

Grotto M, Sulman FG 1967. Modified receptacle method for animal analgesimetry. *Arch Int Pharmacod 165*: 152-159.

Gunatilaka AAL 1996. Triterpenoid quinonemethides and related compounds (Celastroloids). In: Progress in the Chemistry of Organic Natural Products, New York: Springer-Verlag/Wien, vol. 67, p. 1-123.

Hendershot LC, Forsaith J 1959. Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. *J Pharmacol Exp Ther 125*: 237-240.

Janssen PAJ, Niemegeers CJC, Dony JGH 1963. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneimittel-Forsch 13*: 502-507.

Jeller AH, Silva DHS, Lião LM, Bolzani VS, Furlan M, 2004. Antioxidant phenolic and quinonemethide triterpenes from *Cheiloclinium cognatum*. *Phytochemistry 65*: 1977-1982.

Kim IT, Park YM, Shin KM, Ha J, Choi J, Jung HJ, Park HJ, Lee KT 2004. Anti-inflammatory and anti-nociceptive effects of the extract from *Kalopanax pictus, Pueraria thunbergiana* and *Rhus verniciflua*.

J Ethnopharmacol 94: 165-173.

Kutney JP, Hewitt GM, Kurihara T, Salisbury PJ, Sindelar RD, Stuart KL 1981. Cytotoxic diterpenes triptolide, tripdiolide, and cytotoxic triterpenes from tissue cultures of *Tripterygium wilfordii*. *Can J Chem 59*: 2677-2683.

Lião LM, Vieira PC, Rodrigues-Filho E, Fernandes JB, Silva MFGF 2001. Sesquiterpene pyridine alkaloids from *Peritassa campestris*. *Phytochemistry 58*: 1205-1207.

Lião LM, Vieira PC, Rodrigues-Filho E, Fernandes JB, Silva MFGF 2002. Isomeric triterpenoids from *Peritassa campestris*. *Z Naturforsch 57c*: 403-406.

Malone MH 1977. Pharmacological approaches to natural product, screening and evaluation. In: New natural products and plant drugs with pharmacological, biological or therapeutical activity. Springer Verlag, Berlin, p. 24-53.

Medeiros KCP, Monteiro JC, Diniz MFFM, Medeiros IA, Silva BA, Piuvezam MR 2007. Effect of the activity of the Brazilian polyherbal formulation: *Eucalyptus globulus* Labill, *Peltodon radicans* Pohl and *Schinus terebinthifolius* Radd in inflammatory models. *Rev Bras Farmacogn 17*: 23-28.

Milton AS, Wendlandt SA 1970. A possible role for prostaglandin E_1 as a modulator for temperature regulation in central nervous system of cat. *J Physiol-London 207*: 76-77.

Pavanand K, Webster HK, Yongvanitchit K, Kunanake A, Dechatiwongse T, Nutakul W, Bansiddhi J 1989. Schizontocidal activity of *Celastrus paniculatus* wild against *Plasmodium falciparum in vitro*. *Phytother Res 3*: 136-139.

Pinto MN Cerrado 1994. Caracterização, ocupação e perspectivas. Editora Universidade de Brasília, Brasília, p. 345-404.

Puigneró V, Turuli A, Queralt J 1998. Arachidonic acid (AA) and tetradecanoylphorbol acetate (TPA) exert systemic effects when applied topically in the mouse. *Inflammation 22*: 307-313.

Setzer WN, Setzer MC, Hopper AL, Moriarity DM, Lehrman GK, Niekamp KL, Morcomb SM, Bates RB, McClure KJ, Stessman CC, Haber WA 1998. The cytotoxic activity of a *Salacia* liana species from Monteverde, Costa Rica, is due to a high concentration of tingenona. *Planta Med 64*: 583.

Sokal RR, Rohlf FJ 1981, Biometry: The principles and practice of statistics in biological research. 2nd ed. WH Feeman & Co, New York.

Vane JR 1971. Inhibition of prostaglandins biosynthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol 231*: 232-235.

Vane JR, Botting RM 1996. Mechanism of action of anti-inflammatory drugs. Scand. *J Rheumatol 102*: 9-21.

Xie W, Robertson DL, Simmons DL 1992. Mitogen-inducible prostaglandin G/H synthase - a new target for nonsteroidal anti-inflammatory drugs. *Drug Develop Res 25*: 249-265.

Zanini JC, Medeiros YS, Cruz AB, Yunes RRA, Calixto JB 1992. Action of compounds from *Mandevilla velutina* on croton oil-induced ear edema in mice. A comparative study with steroidal and nonsteroidal anti-inflammatory drugs. *Phytother Res 6*: 1-5.