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EVALUATION OF ANTINOCICEPTIVE EFFECT OF *PETIVERIA ALLIACEA* (GUINÉ) IN ANIMALS

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Petiveria alliacea *(Phytolaccaceae) is a bush widely distributed in South America including Brazil, where it is popularly known as "guiné", "pipi", "tipi" or "erva-de-tipi". Brazilian folk medicine attributes to the hot water infusion of its roots or leaves the following pharmacological properties: antipyretic, antispasmodic, abortifacient, antirrheumatic, diuretic, analgesic and sedative. The present study has evaluated the alleged effects of* P. alliacea *on central nervous system (CNS), particularly, the sedative and analgesic properties of root crude aqueous extract of this plant in mice and rats. This extract showed an antinociceptive effect in acetic acid – acetylcholine – and hypertonic saline – induced abdominal constrictions, but not in hot-plate and tail flick tests.* P. alliacea *did not produce any CNS depressor effect. Thus its antinociceptive action in animals can be responsible by its popular use as an analgesic.*

Key words: *Petiveria alliacea* – crude aqueous extract – central pharmacological actions – antinociceptive thresholds

An estimative of the World Health Organization (WHO) have showed that approximately 88% of people living in developing countries rely chiefly on traditional medicines, specially plant extracts, for their primary health care needs (Farnsworth et al., 1985). A demonstration of their efficacy, or at least of their ability to relief symptoms, is the establishment of the use of these plants and/or of their extracts based on trial and error over many generations. *Petiveria alliacea* (guiné, pipi), a bush from Phytolaccaceae family, is a plant commonly used in Latin America for several medicinal purposes. Dried branches are used for toothaches in Brazil (Hoehne, 1939; Reitz, 1967; Van den Berg, 1984). The infusion of leaves and stems is employed as diuretic, abortifacient, febrifuge, antirrheumatic, antispasmodic, antihelminthic in various countries (Burlage, 1968; Pio Correa, 1969; Rocha & Bonzani da Silva, 1969; Bandoni et al., 1976; Morton, 1980). A whole plant decoction and other preparations are used to induce abortion in Mexico (Roig y Mesa, 1945), Guyana (Mihalik, 1978), Trinidad (Wong, 1976) and Brazil (Hoehne, 1939; Rocha & Bonzani da Silva, 1969). An infusion of the roots is used for headaches (Reitz, 1967; Cruz, 1979) and stimulant (Conceição, 1982; Balbach, 1986). There are still anedoctal observations of toxic effects at central nervous system level (Silva, 1935; Souza et al., 1987). Other popular uses were revised by Joly et al. (1987).

However, the few systematic pharmacological studies performed to date with *P. alliacea* showed only immunostimulating activity in mice of the constituents of the insaponifiable fraction (Delaveau et al., 1980) and antimicrobial activity (Van Szczepanski et al., 1972). Thus, the present study was carried out to further characterize the pharmacological effects of *P. alliacea*. We have evaluated the central effects of aqueous crude extract of roots of *P. alliacea* in rodents, mainly, its sedative and analgesic properties.

MATERIALS AND METHODS

Plant material – *Petiveria alliacea* was collected in the state of Maranhão, Brazil, by Dr Terezinha Rego, from the Universidade Federal do Maranhão, São Luis, MA, Brazil. An infusion of roots of this plant was prepared and evaporated in a rota-vapor equipment. Dry extract was ressuspended in NaCl 0.9%.

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Animals – Experiments were conducted with Swiss mice of both sexes (20-30 g), housed in groups of 20 per plastic cage and male Wistar rats (250-300 g) housed 6 per cage in a room of controled temperature (22 ± 2 °C) and lighting (lights on from 6:00 to 18:00 h). Animals had free access to food chow and water.

Gross behavioral observation and acute toxicity – Groups of male and female mice (n = 10) were administered with crude aqueous extract (CE) of roots of *P. alliacea* (800 to 8000 mg/kg), p. o. or i. p. route, and gross behavioral observation was assessed at different time periods (5, 15, 60, 120, 240 min, 24 and 48 h). Mortality rate was registered within 48 h.

Locomotor activity – Adult male mice were administered with CE (500, 1000 and 2000 mg/kg, p. o.) of *P. alliacea,* while control groups received 0.9% NaCl p. o. Animals were placed individually in cages lined with 3 photocell units each for 60 min. Ambulation was automatically recorded as the number of light beam interruptions which occurred during 4 consecutive periods of 15 min.

Motor coordination – Male adult mice, previously selected, were treated with CE (500, 1000 and 2000 mg/kg p. o.) of *P. alliacea* and tested on the rota-rod at various time intervals (3 h in 10 min intervals) according to Zelger et al. (1983). The parameters registered were: (a) percentage of animals which fell off the bar within 1 min of observation and (b) time of staying in the bar at the different time intervals.

To evaluate the possible muscle relaxant effect of the CE another group of animals were injected with 30 mg/kg of morphine s. c. 30 min after pretreatment with 500, 1000 or 2000 mg/kg p. o. of CE of *P. alliacea* and the antagonism of Straub tail reaction was recorded during 30 min (Fukawa et al., 1980).

Pentobarbital-induced sleeping time – Male mice were injected with CE (1000 and 2000 mg/kg p. o.) of *P. alliacea.* After 30 min all animals were injected with sodium pentobarbitone (40 mg/kg, i. p.) and the sleeping time was measured by observing the recovery of the righting reflex up to 3 h.

Convulsive threshold – Male mice were given an intraperitoneal injection of saline solution or CE (500, 1000 and 2000 mg/kg p. o.) and 30 min later the animals received pentylenetetrazol (75 mg/kg i. p.) or a maximal transcorneal electroshock (rectangular pulses of 50 mA delivered at 60 Hz for 200 ms through stainless steel electrodes). The latency until manifestation of the first myoclonic jerk induced by pentylenetetrazol and its duration was recorded up to 30 min after treatment. When convulsions were triggered by electroshock, the durations of the hindlimb flexor and extensor components of the seizure were recorded and the extension time/flexion time ratio was used as an index of convulsion severity (Carlini et al., 1973).

Antinociception evaluation

All tests were performed with male mice except hipertonic saline induced abdominal constrictions that was assessed in rats.

Hot-plate and tail-flick tests – Animals treated with 500, 1000 and 2000 mg/kg p. o. of CE of *P. alliacea* were submitted to a hot-plate (56 °C) and tail-flick test. The thermic reaction latencies were registered by stop watches up to 30 sec and 15 sec of cut-off time, respectively.

Writhing test – Animals treated with CE of *P. alliacea* (125, 250, 500, 1000 and 2000 mg/kg p. o.) were injected intraperitoneally with acetic acid 0.6% (0,15 ml/10 g of body weight), acetylcholine hydrochloride 4 mg/kg and hipertonic saline (NaCl 4%). In order to examine the involvement of opioid mechanisms other groups of animals administered with *P. alliacea* 1000 mg/kg p. o. were pre-treated with 1 mg/kg of naloxone s. c. 15 min prior. The number of abdominal constrictions were recorded during 20, 10 and 10 min consecutives, respectively after acetic acid. acetylcholine or saline algesic stimulus.

Statistical analysis – Data were analyzed by analysis of variance (ANOVA) followed by post-hoc tests when necessary. Differences bellow the probability level of 0.05 were considered statistically significant.

RESULTS

Gross behavioral observation and acute toxicity – Following oral administration of root extract at doses ranging from 800-8000 mg/kg the animals exhibited a reduced locomotor activity. Ptosis and ataxia were observed

in mice dosed with 8000 mg/kg. No deaths occurred 48 h after doses of extracts up to 8000 mg/kg.

Locomotor activity – Figure 1 shows the time course of the effects of the extract on locomotion. The root extract at all doses tested produced a significant decrease on locomotor activity. However this effect was not dose-dependent.

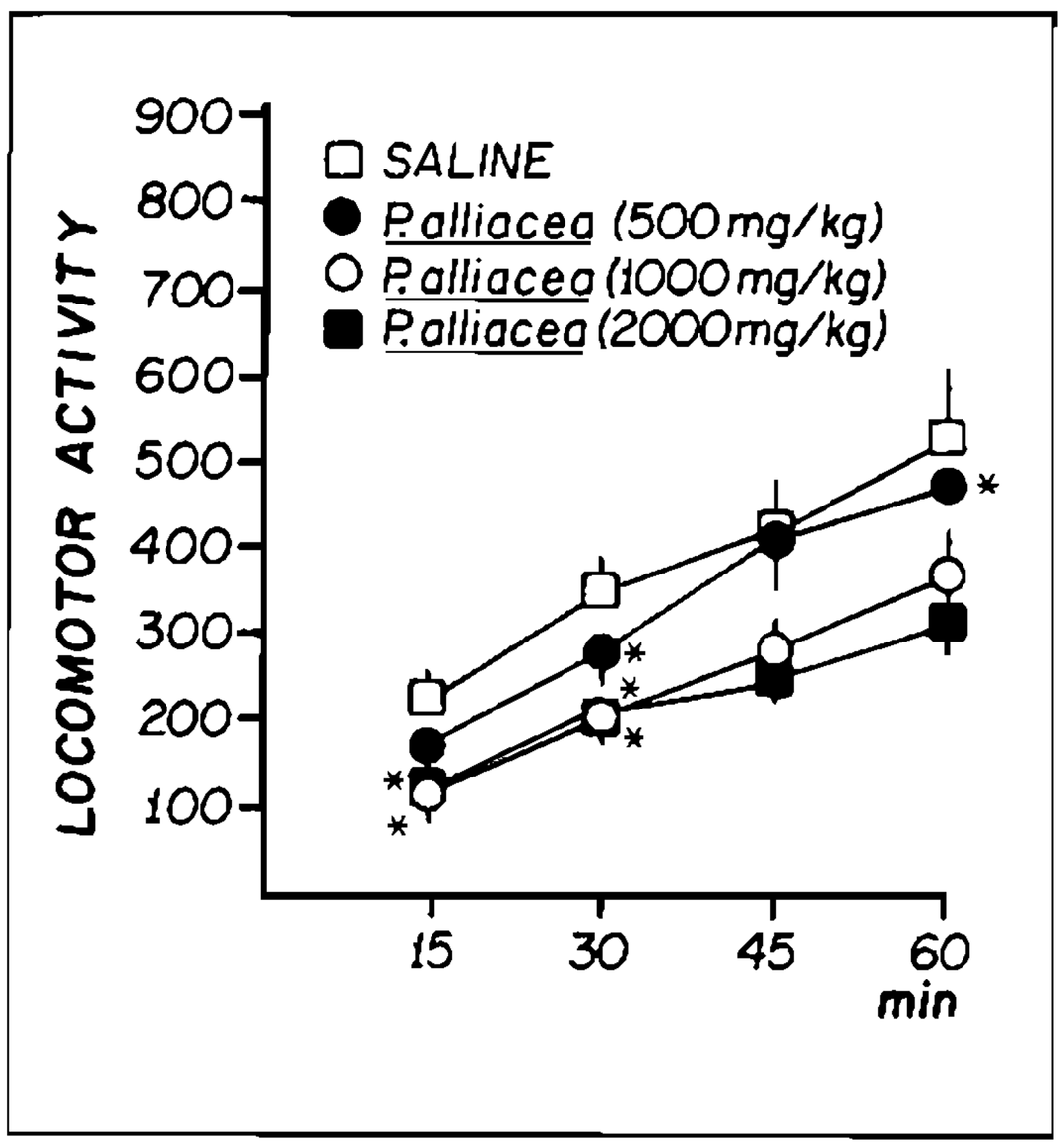

Fig. 1: time course of the effects of the extract of *Petiveria alliacea* on locomotion of mice. Each point represents the mean ± SEM of 10 animals. * $p < 0.05$ compared to control group (two-way ANOVA; followed by Newman-Keuls' test).

Motor coordination – The administration of the extract produced little effect on motor coordination in mice as evaluated in the rota-rod test throughout the experiment (e. g., at 30 min following pretreatment: Controls = 108 ± 12 sec; CE 1000 mg/kg = 90 ± 19 sec; CE 2000 mg/kg = 105 ± 15 sec). This effect was further confirmed by the results on the morphine-induced Straub tail test. The lack of antagonism presented by the extract on the Straub tail in mice suggested an absence of a muscle relaxant effect of the extract.

Pentobarbital induced sleeping-time – Oral administration of the extract did not potentiate the sleeping time induced by pentobarbital in mice (Controls = 3359 ± 362 sec; CE 1000 mg/kg = 3735 ± 625 sec; CE 2000 mg/kg = 4348 ± 553 sec).

Convulsive threshold – Pretreatment with the root extract of *P. alliacea* caused a trend towards an anticonvulsivant effect on pentylenetetrazol-induced convulsions (Table I). Moreover, as shown in Table II, the extract of *P. alliacea* (at 2000 mg/kg) significantly increased the convulsive threshold induced by transcorneal electroshock in mice.

TABLE I

Effects of *Petiveria alliacea* extract on the convulsions and deaths induced in mice by 75 mg/kg pentylenetetrazol i. p.

Dose (mg/kg)	Latency (sec; Mean ± SEM)	Duration	Death (%)
0	270 ± 170	11 ± 1	70
500	148 ± 27	15 ± 1	50
1000	657 ± 254	9 ± 2	30
2000	641 ± 253	8 ± 2	40

TABLE II

Effects of *Petiveria alliacea* extract on flexion time (FT), extension time (ET) and EF/FT relation induced by maximal electroshock (50 mA) in mice. Mean ± SEM

Dose (mg/kg)	FT (sec)	ET (sec)	ET/FT –	Death (%)
0	1,01 ± 0,1	14,0 ± 2,3	12,0 ± 2,6	30
500	1,05 ± 0,3	15,2 ± 1,4	14,6 ± 1,5	30
1000	1,14 ± 0,1	14,3 ± 1,3	15,6 ± 3,0	20
2000	0,98 ± 0,3	7,9 ± 2,2[a]	5,0 ± 1,4[a]	20

a: $p < 0,05$, Dunnett's test.

Antinociception evaluation

Hot-plate and tail-flick tests – All doses of the extract failed to modify the reaction time of mice throughout the experiment as measured by the procedures using thermal nociceptive stimulus, such as the hot-plate and tail-flick methods. Thirty minutes following pretreatment, e. g., the values in hot-plate test were: Controls = 8.9 ± 1.2 sec; CE 500 mg/kg = 9.1 ± 1.7 sec; CE 1000 mg/kg = 7.2 ± 0.6 sec; CE 2000 mg/kg = 8.1 ± 0.8 sec. The values in tail-flick test for the same time period were; Controls = 5.8 ± 1.1 sec; CE 500 mg/kg = 6.3 ± 1.3 sec; CE 1000 mg/kg = 5.7 ± 1.0 sec and CE 2000 mg/kg = 5.1 ± 0.7 sec.

Acetic acid induced abdominal constrictions – Mice treated with the root extract at doses of 500, 1000 or 2000 mg/kg p. o. reduced in a significant way the number of abdominal constrictions induced by i. p. injection of acetic acid. However, this antinociceptive effect of *P. alliacea* was not dose-dependent. Table III summarizes the influence of naloxone pretreatment on the antinociceptive effect of root extract (1000 mg/kg). Curiously, as can be seen the pretreatment with naloxone 1 mg/kg significantly potentiated the action of the extract, while naloxone by itself showed no influence on the number of abdominal constrictions induced by acetic acid (Table III).

TABLE III

Influence of naloxone pretreatment on the antinociceptive effect of *Petiveria alliacea* on writhing test induced by acetic acid 0,6% i. p., in mice

Treatment dose (mg/kg)	Total number of writhes in 10 min Mean ± SEM
Control solution	43,8 ± 2,1
P. alliacea 1000	28,5 ± 3,3[a]
Naloxone 1	42,2 ± 3,6
P. alliacea + NLX	7,5 ± 1,3[a/b]

a: $p < 0,05$ compared to control, Newman-Keuls' test. *b*: $p < 0,05$ compared to *P. alliacea*, Newman-Keuls, test.

Acetylcholine-induced abdominal constrictions – *P. alliacea* root extract (500 and 1000 mg/kg) showed a significant antinociceptive effect as measured by the acetylcholine induced writhing test. As in the preceding experiment, the effect of naloxone pretreatment upon the anti-nociceptive effect induced by *P. alliacea* 1000 mg/kg was assessed (Table IV). Again, the pretreatment with naloxone significantly potentiated the analgesic action of the extract. In addition, naloxone per se showed a significant hyperalgesic action as can be noted by the increase in the number of abdominal constrictions caused by acetylcholine (Table IV).

Hypertonic saline induced abdominal constrictions in rats – Figure 2 shows that the administration of root extract (1000 mg/kg) significantly attenuated the number of writhes induced by i. p. injection of NaCl 4% in rats.

TABLE IV

Influence of naloxone pretreatment on the antinociceptive effect of *Petiveria alliacea* on writhing test induced by acetylcholine 4 mg/kg, i. p. in mice

Treatment dose (mg/kg)	Total number of writhes in 10 min Mean ± SEM
Control solution	8,2 ± 0,5
P. alliacea 1000	5,6 ± 0,9[a]
Naloxone 1	16,1 ± 1,1[a]
P. alliacea + NLX	3,8 ± 0,8[a/b]

a: $p < 0,05$ compared to control, Newman-Keuls' test. *b*: $p < 0,05$ compared to *P. alliacea*, Newman-Keuls, test.

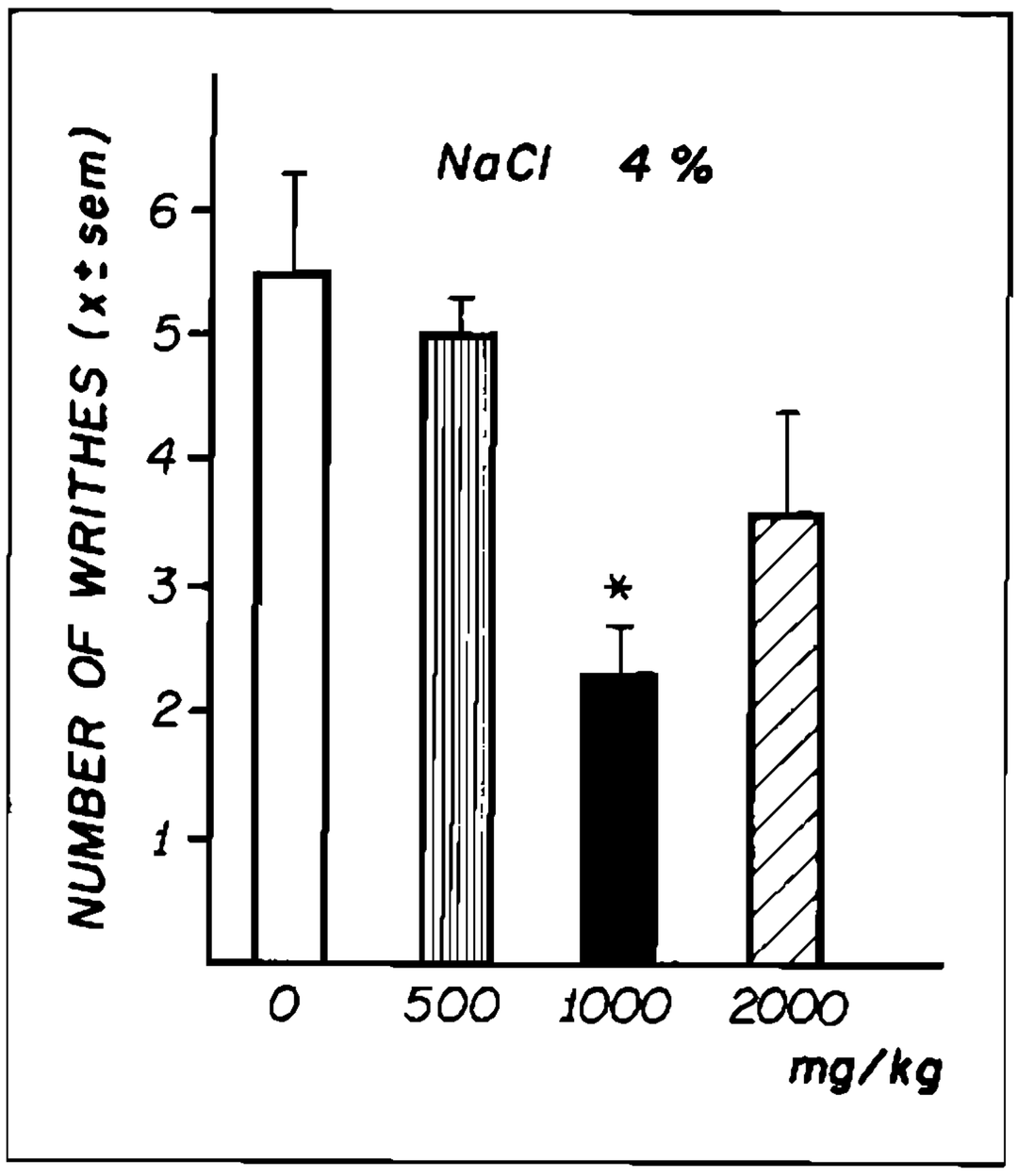

Fig. 2: effects of the extract of *Petiveria alliacea* on hypertonic saline-induced abdominal constrictions in rats. Each bar represents the mean ± SEM of 6 animals. * $p < 0.05$ compared to control group (one-way ANOVA; followed by Dunnett's test).

DISCUSSION

According to the literature, chemical analysis of *P. alliacea* have revealed coumarins (Rocha & Bonzani da Silva, 1969), benzyl-2-hydroxyethyl-trisulfide (Van Szczepanski et al., 1972), sodium nitrate, a mixture of hydrated calcium, potassium and sodium nitrate, allantoin, lignoceric acid, as glutamic acid, serine and glycine in the stems; dibenzyl-trissulfide, glucose and glycine in the roots; alfa-friedelinol, lignoceric

alcohol and lignoceryl lignocerate in the leaves and pinitol in the fruits (Souza et al., 1987). These last compounds may be involved in the central effect of *P. alliacea* in cerebral activity (Souza et al., 1987). However, most of the experiments carried out in the current study to examine a possible CNS depressant-like effect of *P. alliacea* extract did not detect significant changes between control and acutely treated animals. Nevertheless, we did detect a significant reduction in locomotor activity, and an increase in threshold to electroshock-induced convulsions, which could both suggest a depressor action of the extract.

The present results indicate that the pain reaction following i. p. injection of different irritants, such as, acetic acid, acetylcholine or hypertonic saline is attenuated by the oral administration of root extract of *P. alliacea* in mice and in rats.

This antinociceptive effect of *P. alliacea* probably involves peripheral mechanisms, since the extract did not affect the responses elicited by thermal stimuli evaluated by the hot-plate and tail-flick tests, procedures considered most sensitive to detect the central antinociceptive activity (Ramabadram & Bansinath, 1986).

The reversibility of an antinociceptive action by naloxone is widely used to imply a role for endogenous opioid systems. Thus, the present findings showing that the antinociceptive effect of *P. alliacea* is not reversed by naloxone precludes the mediation through opioid mechanisms. Indeed, the fact that naloxone pretreatment enhanced the antinociceptive effect of the root extract as measured by the writhing methods is compatible with a peripheral analgesic site of action. Recently, evidence has accumulated suggesting that peripherally administered opioid agonists or antagonists can produce analgesia in inflammatory conditions (Ferreira & Nakamura, 1979; Stein et al., 1989). Also, it is known that chemically induced nociception can be due to an acute inflammation in the peritoneal area (Deraedt et al., 1980; Gyires & Torma, 1984).

In summary, the present results suggest that the root extract of *P. alliacea* possess antinociceptive action in animals, thus confirming, at least in part, the popular use of this plant to relief pain. Additional experimental series including antiinflammatory and local anesthesic tests are in progress to better characterize the analgesic profile of the extract.

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REFERENCES

BALBACH, A., 1986. *As plantas curam*, 1st ed. EDEL, Itaquaquecetuba, SP.

BANDONI, A. L.; MENDIONDO, M. E.; RONDINA, R. V. D. & COUSSIO, J. D., 1976. Survey of Argentine medicinal plants: folklore and phytochemical screening II. *Economic Botany, 30:* 161-185.

BURLAGE, H. M., 1968. *Index of the plants of Texas with reputed medicinal and poisonous properties* (published by the author).

CARLINI, E. A.; LEITE, JR.; TANNHAUSER, R. & BERARDI, A. C., 1973. Cannabidiol and *Cannabis sativa* extract protect mice and rats against convulsive agents. *J. Pharm. Pharmacol., 25:* 664-665.

CONCEIÇÃO, M., 1982. *As plantas medicinais no ano 2000.* 2nd ed. TAO Editora Ltda., São Paulo.

CRUZ, G. L., 1979. *Dicionário de plantas úteis do Brasil.* Ed. Civ. Brasileira SA, Rio de Janeiro.

DELAVEAU, P.; LALLOUETTE & TESSIER, A. M., 1980. Drogues végétales stimulant l'activité phagocytaire du systeme réticulo-endothélial. *Planta Medica, 40:* 49-54.

DEREADT, R.; JOUQUEY, S.; DELEVALLEE, F. & FLAHAUT, M., 1984. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur. J. Pharmacol., 61:* 17-24.

FANSWORTH, N. R.; AKERELE, O. & BINGEL, A. S., 1985. Medicinal plants in therapy. *Bull WHO, 63:* 965-981.

FERREIRA, S. H. & NAKAMURA, M., 1979. Prostaglandin hyperalgesia: the peripheral analgesic activity of morphine, enkephalins and opioid antagonists. *Prostaglandin, 18:* 191-200.

FUKAWA, K.; LAWANO, O.; HIBI, M.; MISAKI, N.; OHBA, S. & HATANAKA, Y., 1980. A method for evaluating analgesic agents in rats. *J. Pharmacol. Meth., 4:* 251-259.

GYIRES, K. & TORMA, Z., 1984. The use of the writhing test in mice for screening different types of analgesics. *Arch. Int. Pharmacodyn., 267:* 131-140.

HOEHNE, F. C., 1939. *Plantas e substâncias vegetais tóxicas e medicinais.* Depto de Botânica do Estado, Graphicars, São Paulo.

JOLY, L. G.; GUERA, S.; SEPTIMO, R.; SOLIS, P. N.; CORREA, M.; GUPTA, M. LEVY, S. & SANDBERG, F., 1987. Ethnobotanical inventory of medicinal plants used by the guaymi indians in western Panama. Part I. *J. Ethnopharmacol., 20:* 145-171.

MIHALIK, G. J., 1978. Guyanese ethnomedical botany. A folic Pharmacopaeia. *Ethnomedicine, 5:* 83.

MORTON, J. F., 1980. Caribbean and Latin American folk medicine and its influence in the United

States. *Quaterly Journal of Crude Drug Research, 18:* 57-75.

PIO CORREA, M., 1969. *Dicionário das Plantas úteis do Brasil e das exóticas cultivadas.* Min. Agricultura, Inst. Bras. Defesa Florestal, Rio de Janeiro.

RAMABADRAM, K. & BANSINATH, M., 1986. A critical analysis of the experimental evaluation of nociceptive reactions in animals. *Pharmac. Res., 3:* 263-270.

REITZ, P. R., 1967. Fitolacáceas, p. 25-31. In *Flora Ilustrada Catarinense, Parte I.* Itajaí, SC.

ROCHA, A. B. & BONZANI DA SILVA, J., 1969. Análise cromatográfica em camada delgada de alguns princípios ativos de *Petiveria alliacea. Rev. Fac. Farm. Odont. Araraquara, 3:* 65-72.

ROIG Y MESA, J. T., 1945. *Plantas medicinales, aromaticas e venenosas de Cuba.* Ministério de Agricultura, Rep. de Cuba.

SILVA, R. A. O.; 1935. Pipi. *Rev. Flora Medicinal,* 477-487.

SOUZA, J. R.; DEMUNER, A. J.; PEDERSOLI, J. L. & AFONSO, A. M. M., 1987. Guiné: erva medicinal ou tóxica? *Ciência e Cultura, 39:* 646-648.

STEIN, C.; MILLAN, M. J.; SHIPPENBERG, T. S.; PETER, L. & HERZ, A., 1989. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. *J. Pharmacol. Exp. Ther., 248:* 1269-1275.

VAN DEN BERG, M. E., 1984. *Plantas medicinais na Amazônia. Contribuição ao seu conhecimento sistemático.* CNPq/PTU, Belém, PA, Brasil.

VAN SZCZEPANSKI, V. C.; ZGORZELAK, P. HOYER, G. A., 1972. Isolation structure, elucidation and synthesis of an antimicrobial substance from *Petiveria alliacea* L. *Arzneim-Forsch (Drug Res.), 22:* 1975-1976.

WONG, W., 1976. Some folk medicinal plants for Trinidad. *Economic Botany, 30:* 103-142.

ZELGER, K. R. D.; ZELGER, J. L. & CARLINI, E. A., 1983. New anticonvulsivants derived from 4-ally-2-methoxyphenol (eugenol): comparison with common antiepileptics in mice. *Pharmacology, 27:* 40-49.