

ANALGESIC ACTIVITY OF A TRITERPENE ISOLATED FROM *SCOPARIA DULCIS* L. (VASSOURINHA)

SONIA M. F. FREIRE; LUCE M. B. TORRES*; NIDIA F. ROQUE**; CADEN SOUCCAR***
& ANTONIO J. LAPA***/*

Departamento de Fisiologia, *Departamento de Química, UFMA, 65000 São Luis, MA, Brasil **Instituto de Química, USP, 01498 São Paulo, SP, Brasil ***Escola Paulista de Medicina, Departamento de Farmacologia, INFAR, Setor de Produtos Naturais, Rua Três de Maio 100, 04044, São Paulo, SP, Brasil

Analgesic and anti-inflammatory activities of water (WE) and ethanolic (EE) extracts of Scoparia dulcis L. were investigated in rats and mice, and compared to the effects induced by Glutinol, a triterpene isolated by purification of EE. Oral administration (p.o.) of either WE or EE (up to 2 g/kg) did not alter the normal spontaneous activity of mice and rats. The sleeping time induced by sodium pentobarbital (50 mg/kg, i.p.) was prolonged by 2 fold in mice pretreated with 0.5 g/kg EE, p.o. Neither extract altered the tail flick response of mice in immersion test, but previous administration of EE (0.5 g/kg, p.o.) reduced writhings induced by 0.8% acetic acid (0.1 ml/10 g, i.p.) in mice by 47%. EE (0.5 and 1 g/kg, p.o.) inhibited the paw edema induced by carrageenan in rats by respectively 46% and 58% after 2 h, being ineffective on the paw edema induced by dextran. No significant analgesic or anti-edema effects were detected in animals pretreated with WE (1 g/kg, p.o.). Administration of Glutinol (30 mg/kg, p.o.) reduced writhing induced by acetic acid in mice by 40% and the carrageenan induced paw edema in rats by 73%. The results indicate that the analgesic activity of S. dulcis L. may be explained by an anti-inflammatory activity probably related to the triterpene Glutinol.

Key words: *Scoparia dulcis* L – analgesia – anti-inflammatory – medicinal plant

Scoparia dulcis L. is an herb of the Scrophulariaceae family that grows in tropical and subtropical regions, popularly known as “vassourinha”. Medicinal teas of “vassourinha” are used in Brazilian folk medicine for fever and pain relief, in diabetes, menstrual disorders, and respiratory affections (Cruz, 1982; Pio Corrêa, 1984).

We have previously shown that the ethanolic extract of *S. dulcis* L. induced both analgesic and anti-inflammatory effects in mice and rats. Intravenous administration of the same extract to anesthetized rats and cats caused an hypertensive effect that persisted in reserpine treated animals, and was blocked by alfa-blockers (Freire et al., 1985). Chemical purification of the crude extract yielded an organic fraction with analgesic/anti-inflammatory activity, and an aqueous fraction containing the hypertensive principle(s) (Freire et al., 1988).

The present study was undertaken to further analyze the analgesic and anti-inflammatory properties of *S. dulcis* L., and to identify the active principle(s) involved in those effects.

MATERIALS AND METHODS

Extract Preparation – *Scoparia dulcis* L. was collected in the State of Maranhão, Brazil from May through August. The plant was dried at room temperature (28-30 °C), the flowers, leaves and branches were cut, pulverized and stored at 4 °C. The dried plant (20 g) was extracted with 2% hot water (72 °C) for 30 min producing 3 g of a water extract (WE) that was concentrated under vacuum and freeze dried. Ethanolic extraction was obtained from 500 g of *S. dulcis* L. upon mechanic agitation of the mixture at 25 °C for 12 h. This extract (EE) was purified by partition chromatography on silica gel column (hexane, dichloromethane and methanol saturated with water) yielding a mixture of triterpenes in which the active

Financial support from CNPq, CAPES and CEME.

* Corresponding author.

compound was identified by analysis of ^{13}C -NMR and ^1H -NMR spectra as Glutinol.

Pharmacological tests – The experiments were carried out with mice (25-30 g) and rats (180-200 g) of either sex. Pharmacological tests were done with groups of 5 to 10 animals each treated 30 min before by gavage (p.o.) with either plant extracts (WE, EE: 0.5 and 1 g/kg), Glutinol (30 mg/kg) or vehicle (Tween + saline 0.9%) as control. The central nervous system depressant activity was determined by measuring the sleeping time induced in mice after injection of sodium pentobarbital (50 mg/kg, i.p.) (Carlini & Burgos, 1979). The analgesic activity was determined in mice by measuring the pain reaction time to immersion of the tail in a hot water bath (55 °C) (Janssen et al., 1963), and by counting the number of writhes induced by 0.8% acetic acid (0.1 ml/10 g, i.p.) (Koster et al., 1959). The anti-inflammatory activity was tested on the acute rat paw edema induced by a subplantar injection into the hind paw of 0.1 ml of either 1% carrageenan or 1% dextran. The contralateral hind paw was injected with 0.1 ml saline for control. Paw volumes were measured hourly for 2-5 h in a plethysmograph and the swelling was calculated relatively to the initial paw volume (Winter et al., 1962).

Drugs used were: Dextran, Indomethacin, Tween 80 (Sigma, USA), Diphenhydramine hydrochloride (Aldrich, USA), K. Carrageenan (Cialgas, Brazil), Sodium pentobarbital (Abbot, USA), and Acetic acid (Grupo Química, Brazil).

The results were expressed as means \pm s.e. mean and differences among control and treated groups were detected using the Student's "t" test, at a significance level of $P < 0.05$.

RESULTS

Oral administration of WE or EE (0.01 to 2 g/kg) to mice and rats did not induce marked pharmacological effects compared to control animals. After intraperitoneal injection however, only EE caused a proportional decrease of the spontaneous motor activity causing death at high doses (1 g/kg). Previous treatment of mice with either plant extract (0.5 and 1 g/kg, p.o.) however, prolonged the sleeping time induced by sodium pentobarbital by 100% of control values (67.8 ± 12.5 min).

The basal tail flick latency in control mice was 2.4 ± 0.2 s. Those values did not vary over 3 h measurements, and were not altered by pretreatment with either EE or WE (0.5 and 1 g/kg, p.o.). Writhings induced by acetic acid in control mice were 58.9 ± 5.5 writhes/30 min (cumulative counts); previous treatment with 0.5 and 1 g/kg EE, p.o., reduced writhings by 47% and 55% of control. Similarly, in animals pretreated with Glutinol (30 mg/kg, p.o.) writhings induced by acetic acid were decreased by 40% of control values (Fig. 1).

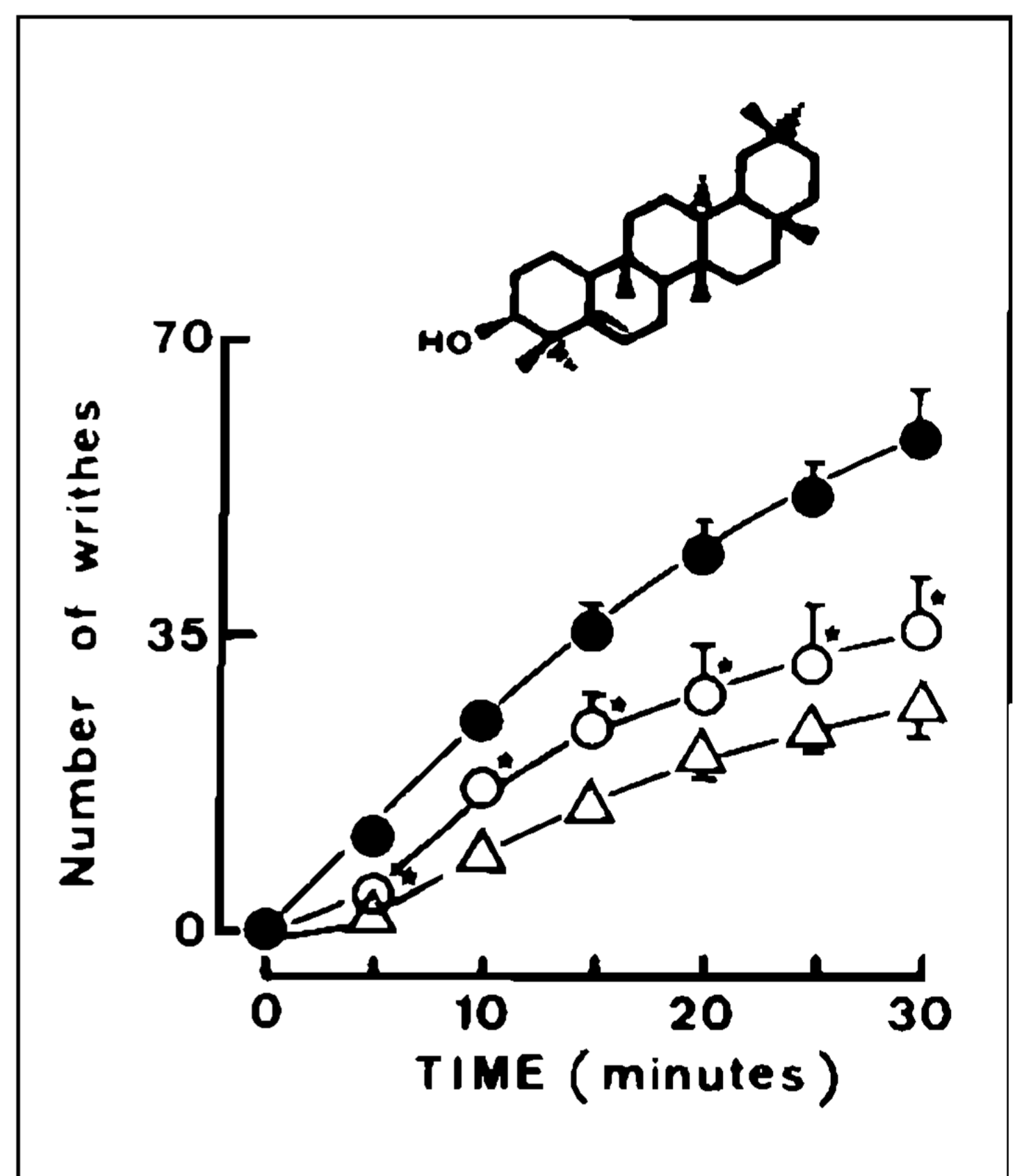


Fig. 1: accumulative number of writhings induced by 0.8% acetic acid (0.1 ml/10 g, i.p.) in control mice (●) and in animals pretreated orally with the ethanolic extract of *Scoparia dulcis* L. (1 g/kg – △) or Glutinol (30 mg/kg – ○). Symbols and vertical bars are means \pm s.e. mean of 8-10 animals. Values marked with asterisk are different from control ($P < 0.05$). Inset – Chemical structure of Glutinol.

Either carrageenan or dextran induced an acute and progressive paw swelling that reached a maximum within 3 or 1 h, respectively (Fig. 2). Neither edema was prevented by treatment of rats with WE (0.5 and 1 g/kg, p.o.). Equal doses of EE, p.o., however, inhibited the carrageenan paw edema by respectively, 46% and 58% after 2 h. Similarly, Glutinol (30 mg/kg, p.o.) inhibited the carrageenan induced paw edema by 73% of control (Fig. 2). Neither EE

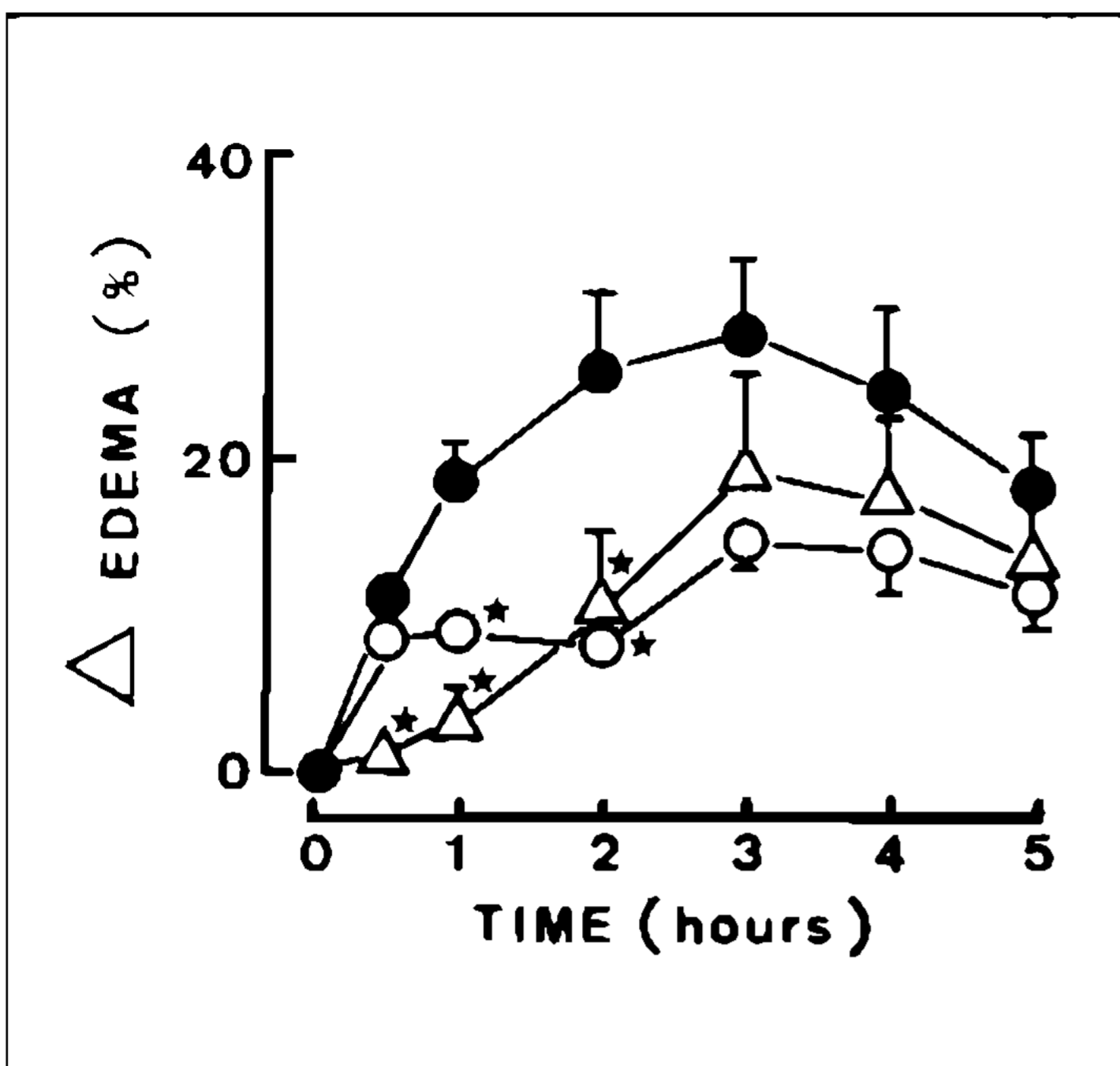


Fig. 2: effects of the ethanolic extract of *Scoparia dulcis* L. (1 g/kg - Δ), Glutinel (30 mg/kg - \circ) and vehicle (\bullet) on the paw edema induced by 1% carrageenan in rats. The differences between volumes of the right paw injected with carrageenan and the left paw injected with equal volume of saline are plotted in ordinates as percentage of the original volumes at time zero. Symbols are means \pm s.e. mean of 5 animals. Values marked with asterisk are different from control ($P < 0.05$).

nor Glutinel treatment influenced the paw edema induced by dextran. Comparatively, in rats pretreated with indomethacin (10 mg/kg, p.o.), the hind-paw edema induced by carrageenan was inhibited by 55% to 80% during 5 h.

DISCUSSION AND CONCLUSIONS

According to the presented data both aqueous and ethanolic extracts of *S. dulcis* L. may depress the central nervous system (CNS), but only the ethanolic extract reduced the pain responses in mice to acetic acid injection. Neither extract influenced the tail flick response in mice, which is a spinal motor reflex sensitive to opioid-like agents (Irwin et al., 1951). The results thus indicate that the analgesic activity attributed popularly to *S. dulcis* L., and previously reported (Freire et al., 1988) is unrelated to the CNS depression induced by both water and ethanolic extracts of the plant.

The ethanolic extract also inhibited the rat hind-paw edema induced by carrageenan, which was not observed with the water extract.

The dextran induced edema was not affected by either extract, suggesting that the analgesic and anti-edema activities of the ethanolic extract are related to the same anti-prostanoid action in the extract. The major active substance isolated from the ethanolic extract was Glutinel, which in parallel experiments was 10 to 15 times more active than the extract itself, and 3 times less active than Indomethacin.

Confirming thus the popular indication, *S. dulcis* L. presents analgesic and anti-edema activities. Our study also indicate that the analgesic effect of the plant is most likely related to the anti-inflammatory activity of Glutinel.

REFERENCES

- CARLINI, E. A. & BURGOS, V., 1979. Screening farmacológico de ansiolíticos: metodologia laboratorial e comparação entre o diazepam e cloro benzapan. *Rev. Assoc. Bras. Psiq. São Paulo*, 1: 25-31.
- CRUZ, G. L., 1982 - *Dicionário de plantas úteis do Brasil*. 2a. Editora Civilização Brasileira, Rio de Janeiro, p. 573.
- FREIRE, S. M. F.; MARQUES, S. G.; SOUCCAR, C. & LAPA, A. J., 1985. Atividade farmacológica da *Scoparia dulcis* L. (Vassourinha). *Cad. Pesq. UFMa*, 1: 39-50.
- FREIRE, S. M. F.; TORRES, L. M.; SOUCCAR, C. & LAPA, A. J., 1988. Fracionamento do extrato etanólico da *Scoparia dulcis* L. com separação dos princípios com atividade analgésica, anti-inflamatória e vasoconstrictora. Resumos do XXXIX Congresso Nacional de Botânica II, p. 50.
- IRWIN, S.; HOUDE, R. W.; BENNETT, D. R.; HENDERSHOT, L. C. & SEEVERS, M. H., 1951. The effects of morphine, methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. *J. Pharmacol. Exp. Ther.*, 101: 132-143.
- JANSSEN, P. A. J.; NIEMEGERES, C. J. E. & DONY, J. G. H., 1963. The inhibitory effect of Fentanyl and other morphine like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneim-Forsch. Drug. Res.*, 13: 502-507.
- KOSTER, R.; ANDERSON, M. & BEER, E. J., 1959. Acetic acid for analgesic screening. *Fed. Proc.*, 18: 412.
- PIO CORREA, M., 1926. *Dicionário das plantas úteis e das exóticas cultivadas*. Ed. 2a., Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro, VI, p. 198.
- WINTER, C. A.; RISLEY E. A. & NUSS, G. W., 1962. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111: 544-547.