Anxiolytic and Sedative Effects of Extracts and Essential Oil from *Citrus aurantium* L.

Maria Isabel Roth CARVALHO-FREITAS and Mirtes COSTA*

Department of Pharmacology, Institute of Bioscience, São Paulo State University (UNESP); 18618–000, Botucatu, São Paulo, Brazil. Received April 15, 2002; accepted August 7, 2002

***Citrus aurantium* L. is commonly used as an alternative treatment for insomnia, anxiety and epilepsy. Essential oil from peel (EOP) and hydroethanolic (70% w/v) extract (HE) from leaves were obtained. Hexanic (HF), dichloromethanic (DF) and final aqueous (AF) fractions were obtained from HE by successive partitions. Swiss male mice (35—45 g) were treated orally with 0.5 or 1.0 g/kg of these preparations 30 min before the experiments for the evaluation of the sedative/hypnotic activity (sleeping time induced by sodium pentobarbital – SPB: 40 mg/kg, i.p.), anxiolytic activity (elevated plus maze – EPM) and anticonvulsant activity (induced by pentylenetetrazole – PTZ: 85 mg/kg, sc or by maximal electroshock – MES: 50 mA, 0.11 s, corneal). The results showed that EOP (0.5 g/kg) increased the latency period of tonic seizures in both convulsing experimental models. This effect was not dose-dependent. Treatment with 1.0 g/kg increased the sleeping time induced by barbiturates and the time spent in the open arms of the EPM. Specific tests indicated that the preparation, in both doses used, did not promote deficits in general activity or motor coordination. HF and DF fractions (1.0 g/kg) did not interfere in the epileptic seizures, but were able to enhance the sleeping time induced by barbiturates. The results obtained with EOP in the anxiety model, and with EOP, HF and DF in the sedation model, are in accord with the ethnopharmacological use of *Citrus aurantium* L., which could be useful in primary medical care, after toxicological investigation.**

Key words anxiolytic; sedative; anticonvulsant; *Citrus aurantium* L.; essential oil; mice

Citrus aurantium L. (Rutaceae), commonly known as sour orange (local name: *laranja-amarga*, *laranja-azeda*, *laranja-cavalo*), is used in Brazilian folk medicine and other countries to treat anxiety,[1)] insomnia,[1—7)] and as an anticonvulsant,[8)] suggesting depressive action upon the central nervous system (CNS), among other properties. Since available pharmacological tools to treat CNS disorders are not disproved from presenting collateral effects, and a significant portion of patients fail to respond to treatments against anxiety and epilepsy, the evaluation of new compounds could be useful in finding safer alternative drugs.

Drug discovery is substantially benefited by ethnopharmacological approaches and, based on their traditional use, preparations obtained from *Citrus aurantium* L. were investigated in order to evaluate their ability to induce sedative/hypnotic, anxiolytic and/or anticonvulsant effects in experimental models.

MATERIALS AND METHODS

Plant Material and Extraction *Citrus aurantium* L. peel and leaves were collected from adult plants in an orchard at the UNESP (Universidade Estadual Paulista) campus from May 2000 to June 2001. A voucher specimen (# 23123) has been deposited in the "Irina D. Gemtchujniov" – BOTU herbarium. Essential oil from peel (EOP) was obtained by steam distillation, then maintained and protected against light and heat until the pharmacological assays. The main component of the EOP was determined by GC-MS to be d-limonene, 90.4%.

Fresh leaves of *Citrus aurantium* L. were detached from the stem and dried at 60 °C/24 h in a hot house with forced circulation and renewal of air. The dried leaves were then pulverized with a mill and the powder kept in a container for extraction by maceration with ethanol 70% (10% w/v) for 48 h. This procedure was repeated three times. After filtration, the residue was discarded and the final filtrate was concentrated in a rotary evaporator (45 °C, under vacuum) to eliminate ethanol. An aliquot of that hydroethanolic crude extract (HE) was separated, and the remainder was subjected to successive extraction with hexane and dichloromethane, resulting in hexanic (HF), dichloromethanic (DF) and final aqueous fractions (AF).

Animals Adult male Swiss mice (35—45 g) were used. All animals were housed in the Central Animal House of UNESP, under standard environmental conditions of temperature, relative humidity and light (24 °C, 60—70% humidity, 12 h light: 12 h dark cycle). Food and water were given *ad libitum* until 2 h before the experimental procedures.

Experimental protocols were designed according to Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA), and were approved by the Bioscience Institute/UNESP – Ethical Committee for Animal Research (CEEA).

Treatments All treatments were administered orally by gavage (vo), and variability in the volume of the administered doses was managed by adjusting the concentration to ensure a constant volume (10 ml/kg). Immediately before use, preparations were suspended in polyoxyethylenesorbitan monooleate (Tween 80—12% v/v in saline, Synth, Brazil), and administered as a single dose expressed as g per kg of bodyweight. The control group received vehicle (Tween) at the same volume as the treated groups. Depending on the experimental procedure (see below), chlordiazepoxide (CDZ – 10 mg/kg: Psicosedin®, Farmasa, Brazil), valproic acid (ACV – 400 mg/kg: Depakene®, Abbott, Brazil) or diazepam (DZP – 1.2 mg/kg: Valium®, Roche, Brazil) was used as the standard drug in order to validate the experimental conditions.

Pentobarbital Sleeping Time Essential oil (EOP at 0.5

* To whom correspondence should be addressed. e-mail: mirtes@ibb.unesp.br

or 1.0 g/kg), extract and fractions (HE, HF, DF and AF at 1.0 g/kg), Tween or CDZ was orally administered, and 30 min afterwards each animal was injected with sodium pentobarbital (SPB – 40 mg/kg, i.p.). The time which elapsed from the injection to the loss of the rightness reflex (induction time) and the time from the loss of rightness reflex to awakening (duration of sleeping) were registered (in seconds) for each animal.[9)]

Elevated Plus Maze Test The elevated plus maze (EPM) consisted of two open arms (15×5 cm) and two closed arms (15×5×12 cm), with the open pair perpendicular to the closed one. The maze was made of clear acrylic and was located 29 cm above a black floor. EOP (0.5 or 1.0 g/kg, vo), extract and fractions (HE, HF, DF and AF – 1.0 g/kg, vo), Tween or DZP were administered, and 30 min afterward, animals were individually placed at the center of the plus maze and observed for 5 min. The time (in seconds) spent by animals in the open and closed arms was registered. Anxiolytic compounds reduced the animal's natural aversion to the open arms and promoted exploration. Therefore, increased time spent in the open arms was considered to reflect an anxiolytic effect, in comparison with the control group, since deleterious effects upon motor activity were not detected in the open field and rota-rod tests.[10)]

Open Field Test The open field area was made of white acrylic (50 cm diameter, 40 cm height walls). The floor had three concentric circles divided into 19 segments of equal area by lines radiating from the center. After 30 min, 2 h and 24 h of treatment with EOP (0.5 or 1.0 g/kg), extracts (HE, HF, DF and AF – 1.0 g/kg) or Tween, the animals were individually placed in the center of the open field. During three minutes of spontaneous ambulation (number of segments crossed with the four paws), the number of rearings, and the time (s) spent frozen (characterized by immobility, with eyes widely open and irregular breathing) or in grooming behaviors was registered.[11,12)] Immediately after observation in the open field, animals were submitted to the rota-rod test.

Rota-Rod Test On a previous day, animals were evaluated in order to select those that had shown ability in walking on the revolving bar under the same conditions used in the test. A non-slippery plastic rod, 3.0 cm in diameter, located 28 cm over the base, formed the apparatus. Immediately after evaluation in the open field test, animals were placed on the horizontal rotating bar of the rota rod (12 rpm). Total time performed on the bar by each mouse during a session (3 successive trials up to 1 min each) was registered using a stopwatch.[13)]

Convulsing Tests The potential to interfere in convulsive processes was studied using pentylenetetrazole (PTZ) and maximal electroshock seizures (MES) in independent groups treated with: essential oil (EOP – 0.5 or 1.0 g/kg), extracts and fractions (HE, HF, DF and AF – 1.0 g/kg), Tween or standard drugs.[14,15)]

Thirty minutes after treatment with these preparations, 85 mg/kg of PTZ dissolved in physiological saline was administered (6 ml/kg, sc. into a loose fold on the dorsal neck). Animals were placed in individual cages and observed for 40 min. The occurrence and latency of clonic and tonic episodes and death were registered for each animal. CDZ was used as the standard drug.

Electric convulsive shock (50 mA, 0.11 s) was delivered bilaterally using corneal electrodes, 30 min after treatments with the preparations. Drops of saline were instilled in both eyes in order to ensure current transmission. The occurrence and latency of tonic convulsion, characterized by hind-limb tonic extension, and death were registered. ACV was used as the standard drug.

Statistical Analysis Quantitative data were compared by parametric (ANOVA, followed by Duncan's test when appropriate) or non-parametric analysis of variance (Kruskal–Wallis followed by Dunn's multiple comparison test when appropriate), depending on the characteristics of the evaluated variable. Proportions were compared by Fisher's exact test. Data from experimental groups were compared with the vehicle group (Tween), and differences were considered significant when $p \leq 0.05$.

RESULTS

Pentobarbital Sleeping Time Data were obtained in seconds and submitted to statistical analysis but, in order to simplify the visualization, the results were transformed into minutes in Figs. 1 and 2. In spite of a similarity of biological effect among the treated groups in Fig. 1, only groups treated with EOP 1.0 g/kg and CDZ produced a significant increase

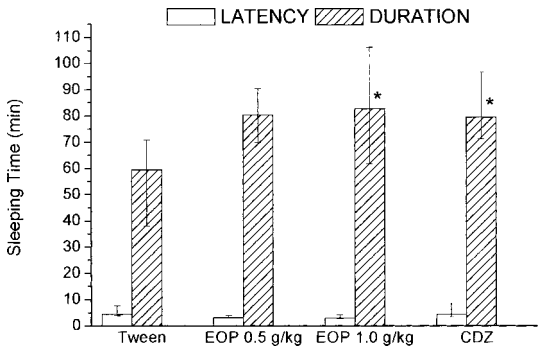

Fig. 1. Effect of Essential Oil on Sleeping Time Induced by Sodium Pentobarbital

Results are expressed as median and interquartile range (Q_1—Q_3). EOP: essential oil, CDZ: chlordiazepoxide (10 mg/kg). Treatments were by oral route. $n=9$ in each group. $*p \leq 0.05$ – Kruskal–Wallis followed by Dunn's test.

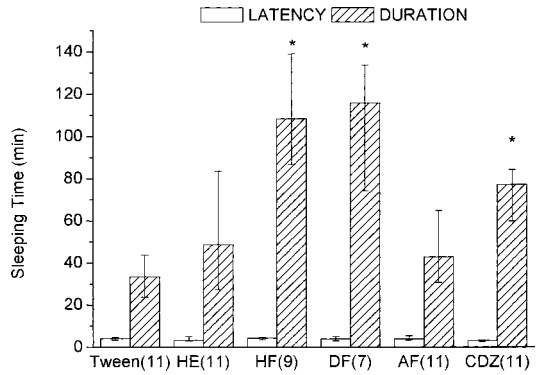

Fig. 2. Effect of Crude Extract and Fractions on Sleeping Time Induced by Sodium Pentobarbital

Results are expressed as median and interquartile range (Q_1—Q_3). HE: hydroethanolic crude extract; HF: hexanic fraction; DF: dichloromethanic fraction; AF: aqueous fraction; CDZ: Chlordiazepoxide (10 mg/kg, *p.o.*). Treatments with extract or fractions were by oral route at 1 g/kg. The number of animals in each group is in parentheses. $*p \leq 0.05$ – Kruskal–Wallis followed by Dunn's test.

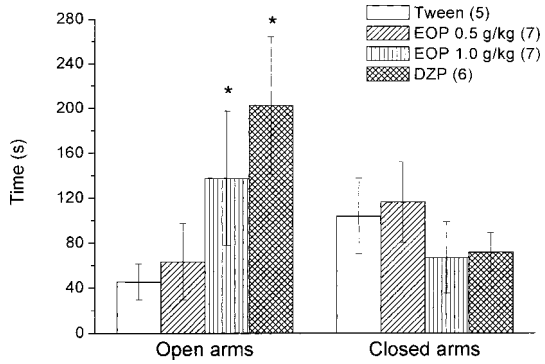

Fig. 3. Effect of Essential Oil on Elevated Plus Maze Test

Results are expressed as mean±standard deviation of time of permanence(s) in open and closed arms. EOP: essential oil (oral route); DZP: diazepam (1.2 mg/kg, i.p.). The number of animals in each group is in parentheses. $*p \leq 0.05$ – ANOVA followed by Duncan's test.

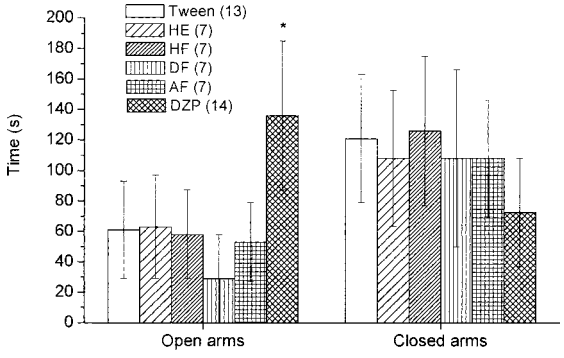

Fig. 4. Effect of Crude Extract and Fractions on Elevated Plus Maze Test

Results are expressed as mean±standard deviation of time of permanence(s) in open and closed arms. HE: hydroethanolic crude extract; HF: hexanic fraction; DF: dichloromethanic fraction; AF: aqueous fraction; DZP: diazepam (1.2 mg/kg, i.p.). Treatments with extract or fractions were made by oral route at 1 g/kg, *p.o.* The number of animals of each group is in parentheses. $*p \leq 0.05$ – ANOVA followed by Duncan's test.

Table 1. Effects on Open-Field and Rota-Rod Tests

Treatment (*n*)	Parameters observed in open field				Performance on rota rod (s)
	Ambulation (Number of times)	Rearing (Number of times)	Grooming (s)	Freezing (s)	
Tween (8)	77 (64—80)	20 (12—26)	17 (9—23)	1.0 (0.0—2.0)	151 (115—171)
EOP 0.5 g/kg (8)	110 (90—122)	23 (17—24)	9 (3—16)	0.5 (0.0—1.0)	156 (85—176)
EOP 1.0 g/kg (8)	78 (52—98)	10 (9—20)	9 (8—13)	0.0 (0.0—1.0)	154 (121—180)
Tween (15)	77 (65—100)	20 (13—27)	9 (3—12)	1 (0.0—1.0)	180 (162—180)
HE 1.0 g/kg (8)	93 (74—96)	25 (17—30)	1 (0—3)	1 (0.5—1.5)	180 (152—180)
HF 1.0 g/kg (8)	91 (67—104)	18 (14—29)	8 (5—16)	1 (0.8—2.0)	173 (121—180)
DF 1.0 g/kg (7)	53 (47—69)	6 (4—12)	14 (4—18)	1 (1.0—3.0)	180 (157—180)
AF 1.0 g/kg (8)	95 (60—107)	23 (17—29)	6 (2—23)	1 (0.8—1.0)	152 (103—166)

Data are reported as median and interquartile range (Q_1—Q_3). EOP: essential oil; HE: hydroethanolic crude extract; HF: hexanic fraction; DF: dichloromethanic fraction; AF: aqueous fraction. Treatments were by oral route.

in hypnotic effect induced by pentobarbital (SPB – 40 mg/kg, i.p.) (Kruskal–Wallis test followed by Dunn's test, $p<0.05$). Despite the tendency toward difference in relation to the Tween group, the results obtained with EOP 0.5 g/kg had no statistical significance when the *a priori* bilateral hypothesis was tested ($p=0.084$). Treatment with 1.0 g/kg of HF and DF (Fig. 2) was effective in significantly enhancing (Kruskal–Wallis test followed by Dunn's test, $p \leq 0.05$) sleeping time induced by SPB. No effect on sleep latency was observed.

Elevated Plus Maze Test Oral administration of 1.0 g/kg of EOP produced a significant ($p \leq 0.05$, ANOVA followed by Duncan's test) increase in permanence in the open arms of the maze (Fig. 3), suggesting an anxiolytic effect of this preparation. However, treatment with the extract and fractions obtained from leaves (HE, HF, DF and AF) did not show any differences from those of the control group (Fig. 4). In both experimental sessions, animals treated with Diazepam (DZP – 1.2 mg/kg, i.p.) spent more time in the open arms of the maze.

Open Field and Rota Rod Tests There were no differences among the treated and Tween groups for parameters observed in the open field (ambulation, rearing, freezing and grooming), or in the rota rod test when evaluated 30 min after treatment with EOP (0.5 or 1.0 g/kg) or with extracts (HE, HF, DF and AF – 1.0 g/kg) (Table 1). The same was observed 2 and 24 h after treatments (data not shown).

These results rule out the possibility of an anxiolytic effect observed with EOP (1.0 g/kg) in the EPM test being due to a motor dysfunction.

Convulsing Test EOP (1.0 g/kg) and extracts (HE, HF, DF and AF – 1.0 g/kg) had no anticonvulsant activity against either PTZ (Table 2) or MES (Table 3)-induced seizures. However, although treatment with EOP 0.5 g/kg did not alter the percentage of occurrence of clonic or tonic episodes or lethality, it increased the latency period until the first tonic convulsion ($p<0.05$; Dunn's Test) in both tests (Tables 2 and 3).

DISCUSSION

Since *Citrus aurantium* L. is popularly used to treat or lighten insomnia and as a sedative,[1—7] the obtained preparations were tested in a pentobarbital sleeping-time model. EOP at 0.5 g/kg showed a tendency to enhance, and at 1.0 g/kg was able to enhance, the sleeping-time duration induced by pentobarbital, as well as HF and DF fractions obtained from leaves. These results suggest sedative/hypnotic activity of *Citrus aurantium* L., which can be useful in cases of insomnia, in accord with their ethnopharmacological use.

It should be pointed out that activity suggested by observation of the enhanced sleeping time may be due to interference with barbiturate enzymatic metabolism. Since there are

Table 2. Effects on Pentylenetetrazole-Induced Seizures

Treatment (*n*)	Clonic		Tonic		Death	
	%	Latency (s)	%	Latency (s)	%	Latency (s)
Tween (10)	100	168 (154—190)	80	706 (398—841)	80	716 (398—858)
EOP 0.5 g/kg (11)	100	184 (166—233)	91	1047 (963—1143)$^{\#}$	91	1047 (963—1156)
EOP 1.0 g/kg (11)	100	211 (160—319)	91	868 (650—1034)	91	877 (662—1034)
CDZ 10 mg/kg (9)	11*	402	0*	—	0*	—
Tween (9)	100	211 (192—300)	78	1033 (818.5—1091)	78	1049 (829—1106.5)
HE 1.0 g/kg (11)	100	368 (208—549)	64	681 (618—948)	64	909 (684—1059)
HF 1.0 g/kg (10)	100	224 (216—352)	90	890 (595—1220)	80	887 (654—1048.5)
DF 1.0 g/kg (10)	100	330 (233—465)	90	1088 (829—1393)	90	1112 (909—1409)
AF 1.0 g/kg (11)	100	336 (208—415)	73	975 (762—1013)	73	993 (770—1181)
CDZ 10 mg/kg (10)	0*	—	0*	—	0*	—

Percentages of occurrence (%) of clonic, tonic and death episodes and their latency reported as median and interquartile range (Q_1—Q_3). EOP: essential oil; HE: hydroethanolic crude extract; HF: hexanic fraction; DF: dichloromethanic fraction; AF: aqueous fraction; CDZ: Chlordiazepoxide. Treatments were by oral route. $^{\#}p \leq 0.05$ – Kruskal–Wallis followed by Dunn's multiple comparison test; $^{*}p \leq 0.05$ – Fisher's exact test.

Table 3. Effects on Maximal Electroshock Induced Seizures

Treatment (*n*)	Tonic		Death	
	%	Latency (s)	%	Latency (s)
Tween (20)	100	2.0 (2.0—3.0)	20	14.5 (13.0—30.5)
EOP 0.5 g/kg (10)	80	3.0 (3.0—4.0)$^{\#}$	0	—
EOP 1.0 g/kg (10)	100	2.5 (2.0—3.0)	20	16.0 (14.0—18.0)
ACV 400 mg/kg (20)	20*	2.5 (2.0—3.0)	0	—
Tween (10)	100	2.0 (2.0—2.0)	20	17.5 (17.0—18.0)
HE 1.0 g/kg (10)	100	2.0 (2.0—3.0)	0	—
HF 1.0 g/kg (10)	100	2.0 (2.0—2.0)	40	19.0 (18.0—22.0)
DF 1.0 g/kg (10)	90	2.0 (1.0—3.0)	40	17.5 (16.5—20.0)
AF 1.0 g/kg (10)	100	2.0 (2.0—2.0)	10	20.0
ACV 400 mg/kg (10)	10*	3.0	0	—

Percentages of occurrence (%) of tonic seizure or death and their latency, reported as median and interquartile range (Q_1—Q_3). EOP: essential oil; HE: hydroethanolic crude extract; HF: hexanic fraction; DF: dichloromethanic fraction; AF: aqueous fraction; ACV: valproic acid. Treatments were by oral route. $^{\#}p \leq 0.05$ – Kruskal–Wallis followed by Dunn's multiple comparison test; $^{*}p \leq 0.05$ – Fisher's exact test.

two isoenzymes of ALT (alanine aminotransferase), one located in the mitochondria and released in the presence of cell necrosis and the other located in the cytosol, which is lost when cell-membrane permeability increases, a change in serum levels of ALT could be interpreted as indicating hepatic damage, especially in rats and mice.[16] In a preliminary approach (data not shown), EOP, HF and DF were not able to alter the enzymatic activity of serum ALT, suggesting an absence of interference with hepatic function.

Among other activities upon the central nervous system attributed to *Citrus aurantium* L., its usage to treat anxiety and hysteria and cases of depression is cited.[1,17] Thus, the preparations were tested by the elevated plus maze test (EPM) in order to investigate this supposed activity of *Citrus aurantium* L. The results showed that EOP (1.0 g/kg) was able to enhance the time spent in the open arms of EPM, suggesting an anxiolytic effect.

This activity detected in EPM could be due to an anxiolytic effect *per se*, or to modifications in exploratory behavior,[18] which were evaluated in the open field test. In this experimental procedure, ambulation and rearing activities are accepted as indicators of exploratory behavior, while grooming and freezing are measures of emotionality.[11,19] Thus, the lack of alteration of these behaviors suggests that EOP does not affect the exploratory behavior or the emotionality. In addition, like the controls, treated animals showed habituation behavior after successive open-field exposure, which was more evident in the exploratory behaviors (ambulation and rearing).[12] In the rota-rod test, animals treated with EOP performed similarly to control animals, suggesting that EOP did not cause damages to motor coordination or equilibrium.[13]

Results obtained in open-field and rota-rod tests indicate that the effect detected in EPM does not indicate a false positive, which would be represented by the incapacity of treated animals to move in the apparatus, suggesting the presence of anxiolytic activity on EOP.

Since protection is represented by the absence of clonic episodes in the PTZ test and of tonic episodes in the MES test,[20] none of the preparations evaluated was able to protect against the convulsive processes. However, the action of EOP (0.5 g/kg) in terms of interfering in seizure episodes could not be discounted, since this preparation elevated the latency of tonic seizures in both experimental models. This effect was not observed with 1.0 g/kg of EOP, confirming the absence of dose dependency.

This kind of biphasic activity is not rare, especially in crude extracts from natural products, constituted by a complex mixture of unknown compounds that could interact in different ways. One probable explanation for this dual effect could be the presence of at least two different substances:

one able to enhance the latency of convulsive episodes while the other presents the opposite activity. At a lower dose of the mixture, the relative amount of the first substance is enough to show a maximal effect, while the amount of the second substance is not enough to show its activity. When animals were treated with the higher dose of the mixture, the amount of the second substance would be enough to originate its effect, overcoming the activity of the first substance and resulting in the absence of effect observed with the lesser dose.

In conclusion, the action of the preparations upon convulsive processes is discreet, but the results obtained with EOP in the anxiety model, with EOP, HF and DF in the sedation model, are in accord with the traditional use of *Citrus aurantium* L., and could be useful in primary medical care after toxicological investigation. In the same way, identification of compound(s) responsible for biological activity could be used as prototype(s) to design new substances with sedative and/or anxiolytic activities.

Acknowledgement We are grateful to Dr. M. Ortiz from IAC (Instituto Agronômico de Campinas) for CG-MS analysis of essential oil from the peel of *Citrus aurantium* L., and to Dr. L. C. Di Stasi for help the in extraction of essential oil. This work constituted part of the M.Sc. Thesis presented to Universidade Estadual Paulista – UNESP, in 2002 by M.I.R.C.F., and was supported by CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPESP (Fundação de Amparo à Pesquisa de São Paulo).

REFERENCES

1) Sanguinetti E. E., "Plantas que Curam," 2nd ed., Rígel, Porto Alegre, 1989.
2) Hernandez L., Munoz R. A., Miro G., Martinez M., Silva Parra J., Chaves P. I., *Am. J. Hosp. Pharm.*, **41**, 2060—2064 (1984).
3) Santos C. A. M., Torres K. R., Leonart R., "Plantas Medicinais (herbarium, flora et scientia)," 2nd ed., Ícone, São Paulo, 1988.
4) Longuefosse J. L., Nossin E., *J. Ethnopharmacol.*, **53**, 117—142 (1996).
5) Germosén-Robineau L., "Farmacopea Caribeña," Emile Désormeaux, Martinica, 1997.
6) Vasquez F. M., Suarez M. A., Perez A., *J. Ethnopharmacol.*, **55**, 81—85 (1997).
7) Gruenwald J., Brendler T., Jaenicke C., "PDR for Herbal Medicines," 2nd ed., ed. by Gruenwald J., Brendler T., Jaenicke C., Medical Economics Company, New Jersey 2000, pp. 346—351.
8) Paul A., Cox P. A., *Econ. Bot.*, **49**, 249—256 (1995).
9) Carlini E. A. "Farmacologia Prática sem Aparelhagem," Sarvier, São Paulo, 1973.
10) Dawson G., Tricklebank M. D., *J. Am. Pharm. Assoc.*, **46**, 208—209 (1995).
11) Archer J., *Anim. Behav.*, **21**, 205—235 (1973).
12) Walsh R. N., Cummins R. A., *Psychol. Bull.*, **83**, 482—504 (1976).
13) Dunham N. W., Miya T. S., *J. Am. Pharm. Assoc.*, **46**, 208—209 (1957).
14) Swinyard E. A., Kupferberg H. J., *Fed. Proc.*, **44**, 2629—2633 (1985).
15) Löscher W., Schmidt D., *Epilepsy Res.*, **2**, 145—181 (1988).
16) Loeb W. F., "Clinical Biochemistry of Domestic Animals," 5nd ed., ed. by Kaneko J. J., Harvey J. W., Bruss M. L., Academic Press, San Diego, 1997, pp. 845—855.
17) Alonso J. R., "Tratado de Fitomedicina: Bases Clínicas y Farmacológicas," ed. by Alonso J. R., Isis Ediciones SRL, Buenos Aires, 1998, pp. 554—571.
18) Rodger R. J., Cao B. J., Dalvi A., Holmes A., *Braz. J. Med. Biol. Res.*, **30**, 289—304 (1997).
19) Hata T., Nishimura Y., Kita T., Itoh E., Kawabata A., *Jpn. J. Pharmacol.*, **48**, 479—490 (1988).
20) Swinyard E. A., Brown W. C., Goodman L. S., *J. Pharmacol. Exp. Ther.*, **106**, 319—330 (1952).