



## Behavioral and enzymatic bioassays with *Serjania erecta* Radlk., Sapindaceae, correlated with cognitive dysfunctions

Luciana S. C. Broggini,<sup>1</sup> Renata S. Fernandes,<sup>1</sup> Thiago Nogueira,<sup>1</sup> Fernanda R. Suzano,<sup>1</sup>  
Ariadiny L. Caetano,<sup>2</sup> Hudson S. Buck,<sup>2</sup> Lucelio B. Couto,<sup>1</sup> Suzelei C. França,<sup>1</sup>  
Paulo S Pereira<sup>\*1</sup>

<sup>1</sup>Unidade de Biotecnologia, Universidade de Ribeirão Preto, Av. Costábile Romano 2201, Ribeirânia,  
14096-900 Ribeirão Preto-SP, Brazil,

<sup>2</sup>Departamento de Ciências Fisiológicas, Faculdade de Ciências Médicas da Santa Casa de São Paulo,  
Rua Dr. Cesário Motta Jr. 61, Santa Cecília, 01221-020 São Paulo-SP, Brazil.

**RESUMO:** “Comportamento e ensaios bioenzimáticos com *Serjania erecta* Radlk., sapindaceae, correlacionados com disfunções cognitivas.” O objetivo deste estudo foi pesquisar o extrato bruto de *Serjania erecta* Radlk., Sapindaceae, e seus bioativos como preventivos ou inibidores de perda de memória em roedores, e outros fatores correlacionados com a síndrome de Alzheimer: atividade antioxidante e anticolinesterásica, principalmente como planta adaptógena, baixa toxicidade e ação regulatória. A reversão do bloqueador colinérgico (escopolamina) no teste da esQUIVA passiva foi detectada pela latência mensurada em animais jovens e adultos. Apresentou baixa toxicidade, com efeito protetor na análise bioquímica (hipoglicemia/hipotrigliceridemia). Índices elevados (acima 83%) na atividade antioxidante foram observados. A inibição da AChE e BuChE foi perceptível nas frações cromatográficas, confirmando as ações via oral e diretamente no SNC.

**Unitermos:** *Serjania erecta*, antioxidante, anticolinesterase, adaptógena, Alzheimer.

**ABSTRACT:** The purpose of this study was to investigate the crude extract of *Serjania erecta* Radlk., Sapindaceae, and its bioactive agents as preventive or inhibitor of memory loss in rodents, as well as other factors correlated with Alzheimer's syndrome: antioxidant and anticholinesterase activity, mainly as plant adaptogen - low toxicity and regulation action. The blocking cholinergic reversion activity (scopolamine) in the test of the passive avoidance was detected by measuring latency in young and adult animals. It presented low toxicity, with protective effect as shown by biochemical analysis (hypoglycemic/hypotriglyceridemic). Elevated levels (above 83%) of antioxidant activity were detected. AchE and BuChE inhibition were also detected in the chromatographic fractions, which were active both orally and directly on CNS (ICV).

**Keywords:** *Serjania erecta*, antioxidant, anticholinesterasic, adaptogen, Alzheimer.

### INTRODUCTION

Proportionally, the age group from the 60s is the one which grows the most. WHO's statistical projections evidence that seniors in Brazil, in the period from 1950 to 2025, should have increased fifteen times, compared with five for the rest of the population. This way, Brazil will be the 6th country regarding the contingent of seniors in 2025, probably having about 32 million people with more than 60 (Cerqueira & Oliveira, 2002).

But considering the level of stress imposed by big cities, the sedentarism, the inadequate nourishment (fast-food, agrotoxics), in addition to the consumption of licit and illicit drugs, the continental groups from the 3<sup>rd</sup> and 4<sup>th</sup> generations are acquiring more and more functional (picture of hysteria, paranoia, depression,

anxiety, hypochondriac cenesthesia) and organic (pictures of demential death or loss of neurons) disturbances, generating new parameters that can lead these individuals to homeostasy and/or heterostasy (Lupien et al., 2005).

Toward this focus, the search for vegetable species that can treat pathologies related to CNS has been explored, mainly for Alzheimer's syndrome, because its pathogeny is unknown and might be unleashed by several factors, isolated or together, as family genetics, neuronal aging, oxidative unbalance (ERO), hormonal dysfunction (still very polemic) or increase in catecholaminergic agents, leading to both cognitive and behavioral alterations (Perry et al., 1998; 1999; Akhondzadeh et al., 2003).

It is well established that the neocortical and hippocampal areas present decreased levels of cholinergic transmission and that there is a loss of neurons from the

\*E-mail address: ppereira@unaerp.br; Tel.: +55 16 3603 6827; Fax: +55 16 3603 7030.

cholinergic nucleus of basal proencephalon and, with evolution of the disease, other neurotransmitters and cerebral regions are affected (Girolami et al., 1996).

Microscopic aspects also are well characterized: amyloid plates consisting of extracellular deposits of amorphous  $\beta$ -amyloid protein and intraneuronal neurofibrillar tangles consisting of filaments of hyperphosphorylated proteins associated to microtubules (TAU) - reflex of an abnormal neuronal cytoskeleton. This entangled material also appears in normal brains, however in smaller amounts (Robbins et al., 1999; Forlenza & Gattaz 1998).

Several researches are being carried out as to detect the pathogens as to diagnose the syndrome in its initial phase - the pathology displays three characteristic phases: amnesic or initial (memory lapses, difficulty with language, time and space disorientation, depression), intermediary or demencial (loss of recent memory, anxiety, aggressiveness, deliriums), advanced or vegetative (late/consolidated memory loss, vegetative state). The diagnosis is imprecise regarding the phase in progress (Jordan-Sciutto et al., 2002; Halks-Miller et al., 2003).

According to Cacabelos (2000) the pathogens can be classified into four categories: primary events (genetic factors, neuronal apoptosis), secondary events (deposition of  $\beta$ -amyloid agents in senile plates and cerebral vessels, neurofibrillar tangles due to hyperphosphorylation of the TAU protein, synaptic decrease), tertiary events (decrease of neurotransmission, neurotrophic alterations, neuroimmune dysfunction, neuroinflammatory reactions) and quaternary events (excitotoxicity reactions, alteration in the calcium metabolism, formation of free primary radicals or vascular brain reactive species), addressing screening *in vitro* and *in vivo* with bioactive drugs.

One of the medicines from first choice in the treatment contains the active principle galanthamine (Remynil®), present in *Galanthus* (snow drop) and wild *Narcissus* plants, with nicotinic agonist and ACh (acetylcholine) liberation, as well as cholinesterase actions (inhibition of AChE) (Stahl, 1996). The pharmaceutical approach used up to now causes only a low progression of the disease, and it presents serious side effects to an already weakened patient, justifying the search for new substances that can embrace these factors, or part of them.

Other factors that can evoke further reactions involve free radicals. When in balance, their production and elimination do not cause cellular damages. But when generated in excess, decurrent of several factors as chemical lesion or radiation, oxygen and other gases, cellular aging, microbial death of phagocytic cells, inflammatory damage, tumoral destruction of macrophages, they caused deterioration, many times irreversible (Cotran et al., 1996). Several medicinal plants own active principles with antioxidant activity, mainly those that contain phenolic compounds, it substances that can lead to the stagnation of these harmful events to the organism (Mori & Hasegawa, 2003).

The present investigation on *Serjania erecta* Raldk. (Sapindaceae – class *Serjania* Mill) is based on its popular use: inflammation, stomach ache, ulcerative diseases, and hypertension (Guarim Neto et al., 2000, Pott et al., 1994) and to the compound class that Sapindaceae contains: saponin, plant adaptogen marker, with well-known depurative, tonic-stimulant and immunomodulator actions, acting as preventive and/or memory loss inhibitor in rodents, in addition to other factors correlated with Alzheimer's syndrome - oxidation of metabolites and action of cholinesterases, with low toxicity and as organic regulator (Mattei et al., 1998). Pharmacological evaluation of *S. erecta* showed topical anti-inflammatory activity in mice (Goming et al., 2008) and gastroprotective activity in rats (Arruda et al., 2009). Goming et al. (2008) showed the presence of saponins, flavonoids, triterpenoids, steroids, tannins, and catechins in stems and leaves from *S. erecta* ethanol extract.

## MATERIALS AND METHODS

### Animals

The animals were obtained from Unaerp's biotery, that meets with all the requisites for laboratory animals handling and creation of Cobeia (Brazilian School of Animal Experimentation). The bioassays procedures used, were approved by the Ethics Committee of Unaerp, which follows the rules recommended by International Guiding Principles for Biochemical Research Involving Animals (CIOMS). Young male and female Swiss albino mice (3-4 months old; 18-25 g), and young (3-4 months old; 150-180 g) and adult (11-12 months old; 450-500 g) male Wistar rats were used, housed in plastic boxes in groups of five, under controlled temperature (23±1 °C) and light-dark cycle of 12 h. Water and food were supplied *ad libitum*.

### Plant and drugs

The plant was collected at Medicinal Plants Collection of Unaerp and it was identified by Dra. Inês Cordeiro, Botany Institute (São Paulo-SP, Brazil). A voucher specimen was deposited under the number HPMU-835 at Herbarium of the Biotechnology Department of Unaerp (Ribeirão Preto-SP, Brazil). Drugs, such as rutin, albumin, galanthamine, Tris-HCl, DPPH, acetylcholinesterase, butyrylcholinesterase, salt fast blue B and 1-naphthyl acetate were acquired from Sigma®; scopolamine was from Fluka®.

### Chromatography

Silica-gel G plates and Sephadex LH20 were purchased from Aldrich®, LC18 column (HPLC) from Supelco®. Solvents, including methanol, hexane, ethyl acetate, formic acid and glacial acetic acid were obtained from Synth®. HPLC analyses were carried out using a reverse phase column (Supelcosil™ RP-18, 250 x 4.6

mm i.d.), monitored at 220, 254 and 350 nm. A two-solvent gradient system of water:acetic acid 0.1% (A) and MeOH (B) was used. The gradient program consisted of three periods: (1) 0-32 min, 10-66 % B, (2) 32-35 min, 66-10 % B, (3) 35-40 min, 10 % B. The flow-rate was 1.0 mL/min and the sample injection volume was 20 µL. TLC analyses were performed using silica gel plates (10x 10 cm or 10x 5 cm). Two solvent systems were used: (A) CHCl<sub>3</sub>:MeOH (4:1) and (B) BAW (n-butanol:acetic acid:water, 4:1:5; upper phase) and the color reagents were NP-PEG and vanillin-sulfuric acid.

### Extractive processes

After the collection, the material was separated in its parts - leaf, stem and root, which were dried in a stove at 50 °C (enzymatic stabilization) for 72 h (leaf) and 96 h (other parts), and triturated in a knife mill (drug pulverization with thick cut to 50 mesh). The resulting ground from leaves (90 g) was submitted to extraction with distilled ebullient water (900 mL) and then left standing for 24 h in the dark. Aqueous extract was filtered and lyophilized (Flexi-Dry™), yielding 13 g for the bioassays and purification, and the dry residues kept in a stove for 72 h, for posterior extractions with MeOH and CHCl<sub>3</sub>. Aqueous extract (2 g) was submitted to partition and division into fractions. The residues were resuspended in MeOH:H<sub>2</sub>O (85:15; 50 mL), extracted with hexane (three extractions x 50 mL), the hydroalcohol fraction being concentrated and again partitioned with ethyl acetate (three extractions x 50 mL). All fractions were concentrated on a rotating evaporator (Marconi®). The hydroalcohol fraction (1.7 g) obtained was resuspended in methanol and applied on a Sephadex LH20 column (1.8 x 40 cm) yielding fractions 1 (626.4 mg), 2 (223.7 mg), 3 (621.3 mg; saponin fraction), 4 (84.1 mg; saponin fraction), 5 (64.6 mg; flavonoid fraction) and 6 (35.8 mg; tannin fraction) (Fig. 1). Some fractions were applied on a preparative HPLC column (Shimadzu®) using a reverse phase column (Supelcosil™ RP-18, 250 x 10 mm i.d.), monitored at 210, 254 and 350 nm. A two-solvent gradient system of water (A) and MeOH (B) was used. The gradient program consisted of three periods: (1) 0-100 min, 0-100 % B, (2) 100-105 min, 100-0 % B, (3) 105-110 min, 0 % B. The flow-rate was 2.0 mL/min and 4 mL to fraction collection. All fractions were monitored by TLC until purification [TLC solvent systems: CHCl<sub>3</sub>:MeOH (4:1) and BAW (n-butanol:acetic acid:water, 4:1:5; upper phase); TLC color reagents: NP-PEG, vanillin-sulfuric acid, and Dragendorff reagents].

### Acute toxicity and screening behavioral

Acute toxicity studies were performed according to RE 90 of ANVISA (National Agency of Sanitary Vigilance) using 100 male and female Swiss mice (*i.p.* and *p.o.*) and the behavioral screen according

to the protocol described by Almeida et al. (1999), using fifty male Swiss mice under several dosages.

### Cognitive evaluation

The memory evaluation was accomplished in an equipment named passive avoidance (Insight®), an acrylic box composed by an illuminated compartment (aversive factor) linked through a door to a dark compartment with a parquet of alternated metallic bars (situation factor, with trauma provoked by 5 foot shocks of 0.8 mA/2 s), similar to the one described by Angelucci et al. (1999) The training session (latence of acquisition) consisted in treating the animal (3 and 11 months old male Wistar rats) with a crude extract for seven days (50 mg/kg *p.o.* distilled water as solvent) and scopolamine (30 mg/kg *i.p.* dissolved in NaCl 0.9% - negative control) 30 min before putting him in the light compartment, using galanthamine (30 mg/kg *i.p.* dissolved in NaCl 0.9%) as positive control in the aged animals. The retention test was accomplished after 24 h, measuring the maximum time of 180 sec in which the animal moves from the clear compartment to dark (Espínola et al. 1997). After the test, autopsy of the organs was performed, followed by hematological (blood with EDTA, ABC VET, ABX) and biochemical (Kinetic for automation, Cobas Aim Plus CC) analyses, diagnosed by the Provet (São Paulo-SP, Brazil) and Analyses Clinics Laboratory of Unaerp (Ribeirão Preto-SP, Brazil).

### Intracerebroventricular cognitive evaluation (ICV)

Male Wistar rats were anesthetized with equitezim (1 mL/100 g *i.p.*) prepared as follows: cloral hydrate, magnesium sulfate, propilenglycol, absolute alcohol, distilled water and thionembutal. After sedation, it was hold by the auricle in a stereotaxic equipment, for depilation and identification of the cranial bregma. From this point on, the antero-posterior (AP 0.8 mm), lateral (L 1.5 mm) and vertical (V 3.5 mm) points was localized, according to the coordinates of Paxinos & Watson (1998). In sequence, a stem of 0.8 mm, two screws and an acrylic autopolymerizer were introduced. After 72 h recovery, 5 µL each of the active fraction SS (*Serjania* saponins, 0.5 mg/mL NaCl 0.9%) and scopolamine (30 µg/kg NaCl 0.9 % *i.p.*), 1 h and 30 min, respectively, before the passive avoidance test (0.8 mA shock/5 s), were injected.

### Antioxidant evaluation

The antioxidant activity was evaluated at 517 nm by reduction of 10<sup>-4</sup> M DPPH (2,2-diphenyl-1-picrylhydrazyl) using spectronic® spectrophotometer, through inhibition of the formation of free radical by the crude extract (SE10, 10 mg/mL MeOH; SE5, 5 mg/mL MeOH; SE2.5, 2.5 mg/mL MeOH) and by the semipurified fractions (tannin, 0.1 mg/500 µL MeOH; saponin, 2.0 mg/500 µL MeOH;

flavonoid, 1.9 mg/500  $\mu$ L MeOH), using rutin as positive control (10 mg/mL MeOH) and, as negative control, 1 mL of DPPH with 50  $\mu$ L MeOH. After incubation (50  $\mu$ L with 1 mL DPPH, in triplicate) at 30 °C for 20 min, the reading and calculation of the % inhibition absorbancies were made  $[(Ab-AA)/Ab] \times 100$ , where Ab is the control absorbance and AA the sample absorbance (Koleva et al. 2002).

### Enzymatic evaluation

The bioautographic test developed by Marston et al (2002) is based on the acetate cleavage of 1-naphthyl by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) to form the 1-naphthol which, reacting with salt fast blue B, will color TLC plates in purple, originating from formed diazon. The enzymatic inhibition will be visible through the formation of colorless and yellow halos on the silica plates. For the analytic method, 1000 IU AChE were dissolved in 150 mL of Tris-HCl (0.05 M; pH 7.8) buffer, followed by 150 mg of bovine serum albumin (protean stabilizer). After treating the TLC plate with acetone, 15  $\mu$ L of each sample (tannin fraction, 0.1 mg/100  $\mu$ L MeOH; flavonoid fraction, 1.9 mg/500  $\mu$ L MeOH; saponin fraction, 2.0 mg/500  $\mu$ L MeOH; crude extract, 10 mg/mL MeOH) were applied. The plate was eluted with CHCl<sub>3</sub>:MeOH (4:1) and after drying, pulverized with the enzyme solution previously prepared and incubated in a wet container at 37 °C by 20 min. For development 10 mL of naphthyl and 40 mL of fast blue B were used. The experiment was repeated with 500 IU of BuChE under the same conditions.

### Statistical analysis

The results are expressed as means $\pm$ S.D. Statistical significance was determined by ANOVA, followed by a post hoc Tuckey test. A probability *p* value of less than 0.05 was considered to indicate statistical significance.

## RESULTS

### Purification of bioactive compounds

In the leaves aqueous extract from 90 g dried plant, some bioactive compounds with well-known pharmacological actions were identified, such as: saponins fraction (4.7 mg, vasoprotector, antiedematogenic and tonic-stimulant), tannins fraction (1.4 mg, healing, antiinflammatory and antiulcerogenic) and glucosidic flavonoids fraction (7.2 mg, antioxidant, antiulcerogenic and antiinfeccion). Sephadex LH 20 column was important for purification of the ethanolic fraction, providing a six-group profile: fractions 2 and 5 showed flavonoids in its composition; fractions 3 and 4 showed saponins; and fraction 6 showed tannins. (Figure 1). HPLC profile of the crude extract showed five flavonoids as main compounds (Figure 2A). Fraction 2 presented one flavonoid with retention time

23.3 min (spectrum data similar to rutin spectrum), while fraction 5 presented four compounds: two with spectral data similar to rutin ( $t_R = 25.9$  and 27.5 min) and other flavonoid compounds ( $t_R = 22.6$  and 24.6 min) (Figure 2B). At least, three saponins were visualized in TLC (Figure 1B). The fractions are being purified and they will be submitted to HPLC-MS, RMN 1H and <sup>13</sup>C for elucidation of structures.

### Screening behavioral and acute toxicity

Screening made with the crude extract in male Swiss mice presented behavioral effects from the dose of 500 mg/kg *p.o.* and side effects from the dose of 1000 mg/kg *i.p.*, without any death (Table 1). Behavioral screening was made for 72 h with the aqueous extract from the leaves, root and stem, selecting the leaves extract due to its activity *in vivo*. In the toxicity assay, evaluated for fifteen days, mice from both sexes presented low *i.p.* toxicity, as indicated by the numbers of deaths and abdominal contractions from the dose of 1250 mg/kg.

**Table 1.** Screening behavioral in several administration doses and vias with Swiss mice observed by 72 h (n = 5 for group/via; *p.o.* control: distilled water; *i.p.* control: physiologic serum).

Administration via	Doses (mg/kg)	Main observed behavioral effects
Intraperitoneal	312	without effect
	625	piloerection
	1250	reduced muscular force, ataxia, ambulation decreased, female death
	2500	tremor, abdominal contortion, male death
	625	piloerection, rearing, smell intermittent, vocalization,
Oral	1250	climb
	2500	stereotypy
	5000	force to grab, panting breathing

### Cognitive evaluation

The aged animals treated for seven days (50 mg/kg *p.o.*) obtained latency of 73 s when compared with the group that received only galanthamine (30  $\mu$ g/kg *i.p.*, 94 s) and to the scopolamine group (30  $\mu$ g/kg *i.p.*, 57 s) in the memory retention evaluation for 24 h (Figure 3). Under the same conditions, the young animal presented more accentuated results, obtaining latency of 176 s when compared with the group that received only scopolamine - latency of 32 s (Figure 4).

After the test, acute-subchronic toxicity was evaluated, no body weight variation being detected, in addition to no alteration of the tissue and weight of the organs (Table 2), ulcer absence that could have been induced by the shocks in the passive avoidance. The post-test biochemical analysis showed protective



effect in the glycemic rate of the adult animals and in triglyceride evaluation in young and adult animals (Table 3). The biochemical analysis of more expressive toxicologic metabolites (renal and hepatic) also showed normal parameters for the evaluated species (Wistar rats), without toxic signals (Table 4). The hematologic analysis also presented normal parameters in quantity and morphology (white and red series, platelet) (Table 5).

**Table 2.** Acute/subchronic toxicity evaluated in male Wistar rats treated with 50 mg/kg of *Serjania erecta* (SE50) for seven days.

Groups	Control (H <sub>2</sub> O)	Treated (SE50)
<i>Variation of Corporal Mass</i>		
Treatment start	191.20±18.60	187.40±16.20
Treatment final	225.60±23.50	217.10±21.30
<i>Evaluation in the weight of the organs</i>		
Heart	1.20±0.30	1.00±0.00
Liver	12.50±0.70	9.70±1.40
Kidneys	2.20±0.30	2.00±0.30

Expressed values means±S.D. (n=10). Evolution ponderal: ANOVA/Tukey  $p<0.05$ . Alteration in the weight of the organs: ANOVA/Tukey  $p>0.05$

**Table 3.** Biochemical analysis in young and adult animals in the dose of 50 mg/kg of *S. erecta* extract.

Analyses	Glucose	Cholesterol	Triacylglycerides
<i>Control group (H<sub>2</sub>O)</i>			
Young rodents	67.20±16.21	60.67±6.74	80.00±34.04
Adult rodents	84.00±23.30	73.00±6.20	82.70±21.40
<i>Treaty group (SE50)</i>			
Young rodents	77.00±13.52	55.50±3.51	67.00±26.46
Adult rodents	68.90±23.30	71.20±14.40	62.90±14.40

Expressed values means±S.D. (n=10) ANOVA/Tukey  $p>0.05$ .

**Table 4.** Biochemical analysis in young animals (4 months).

Analyses	Control (H <sub>2</sub> O)	Treated (50 mg/kg)
Creatinine	0.41±0.36	0.75±0.14
Urea	39.52±2.28	41.72±8.29
AST	59.85±19.84	66.56±4.28
ALT	24.35±4.17	21.55±4.54

Expressed values means±S.D. (n=10) ANOVA/Tukey  $p>0.05$

### Cognitive evaluation ICV

The animal's treated acutely via ICV with the saponin (SS) fraction reverted the cholinergic blocking (scopolamine) with latency of 51 s when compared with the scopolamine group (19 s) (Figure 5).

### Antioxidant activity

The flavonoid (88%) and tannin (83%) fractions, and the crude extract SE10 (94%), SE5 (91%) and SE2.5 (92%) presented themselves as antioxidant *in vitro* when compared to the rutin standard (92%). Even the saponin fraction, for which this activity is not described in the literature, showed to be antioxidant with inhibition of 18%, indicating potencialization by the *S. erecta* crude extract (Figure 6).

### Enzymatic activity

The results demonstrated that the sequential time to substance-enzyme-discloser interaction (15 to 1440 min) provided larger clearness of the enzymatic inhibition, suggesting that the pharmacological action is above 24 h. Flavonoid fraction presented better resolution, being more selective for AChE than to BuChE. Saponin fraction was better visualized in the first assay (not demonstrated) and after 24 h in the second one, indicating larger inhibition for BuChE than for AChE.

**Table 5.** Hematologic parameters in young animals (4 months).

Hemogram	Control group	Treaty group	Reference
Erythrocytes	6.8±0.9	6.8±0.2	8.3±1.1 mil/mm <sup>3</sup>
Hemoglobin	13.2±0.8	13.4±0.5	15.0±1.4 g/dL
Hematocrit	37.8±2.9	38.2±0.4	47.0±3.5 %
VCM	56.0±3.6	56.7±1.5	57.5±7.8 u <sup>3</sup>
HCM	19.6±1.7	19.8±0.7	18.5±2.1 pg
CHCM	35.2±0.8	35.0±1.4	32.3±2.5 g/dL
Total protein	6.7±0.7	6.9±0.2	6.2±0.5 g/dL
Erythroblasts	0.0	0.0	0.0 %
Reticulocytes	0.0	0.0	2.5±2.1 %
Leukocytes	2.6±0.4	3.1±0.9	8.5±3.4 mil/mm <sup>3</sup>
Medulla cells	0.0	0.0	0.0 %
Metamyelocytes	0.0	0.0	0.0 %
Short sticks	0.0	0.0	0.0 %
Segmented	24.7±3.8	18.3±2.1	21.5±17.7 %
Eosinophil	0.3±0.6	0.3±0.6	3.0±4.2 %
Basophilic	0.0	0.0	1.0±1.4 %
Typical lymphocytes	74.3±3.1	81.0±1.0	75.0±14.1 %
Atypical lymphocytes	0.0	0.0	0.0%
Monocytes	0.7±0.6	0.3±0.6	2.5±3.5 %
Platelet count	1.1±0.2	1.2±0.1	1.0±0.3 mil/mm <sup>3</sup>

Expressed values means±S.D. (n=10) – ANOVA/Tukey  $p>0.05$

### DISCUSSION

Recently, the medicinal plants, first considered from secondary-rate, came back into vogue with the confirmation of important pharmacological actions

and of an excellent cost-benefit relationship. Based on ethnopharmacology, the search for new substances and development of new medications has shown itself more promising, with physiologic actions and more addressed targets, in addition to smaller toxicity and side effects.

To mediate the studies in CNS, Carlini (2003) described medicinal plants with neurological effects known as psychodysleptic (hallucinogens, *Cannabis sativa*, *Tabernanthe iboga*), psycholeptic (analgesic or anxiolytic, *Piper methysticum*, *Valeriana* spp, *Passiflora incarnata*) and psychoanaleptic (stimulants, *Catha edulis*, *Paullinia* spp, *Ephedra* spp), leading to a functional framing in the research of this segment.

The concept of adaptogen or resistogen plants is included in the psychoanaleptics class: "Plants that increase the organism resistance, in stress situations, without leading to the loss of the internal homeostas", that is, without causing exhaustion provoked by the stimulants. The answer of adaptogens to stress is independent of the nature of the stimulus (chemist, physical or biological) and nonspecific (Panossian et al 1997; Panossian et al 1999; Singh et al 2003). Davydov & Krikorian (2000) described that this category presents antioxidant, immunomodulatory, hypoglycemic and hypocholesterolemic activity, being used by millenary Chinese medicine (Ma Huang and Huangki) and ayurvedic (rasayanas), some prescribed also by the Western medicine as *Ginko biloba*, *Panax ginseng* and *Echinacea purpurea* (Dahanukar & Thatte, 1997; Howes & Houghton 2003). Researches demonstrated cognitive improvements (learning and memory) with significant results in the acquisition, consolidation and evocation of memory (Kidd, 1999; Siripuraru et al. 2005), improvements in the sleep quality and in the sexual performance (Brekhman & Dardymov, 1969; Baranov, 1982) as well as normalization of acute and chronic stress in rodents (Rai, 2003).

The neotropical class *Serjania* Mill displays about 230 well-known species, being the most numerous of this tribe (Ferruck & Acevedo-Rodriguez, 2005). In this group, is found the family Sapindaceae, rich in saponins, active principle characteristic of adaptogens plants, such as *Paullinia cupana* and *Cardiospermum halicacabum*, with well-known depurating, tonic-stimulant and immunomodulating actions (Mattei et al 1998).

According to our results, the species *S. erecta* was able to revert the cholinergic blocking with significant latency, and low toxicity in the treatment for seven days, with protective effects shown by the biochemical analyses, demonstrating that it can act as adaptogen at 50 mg/kg. DL50 data showed that it led to death at 1250 mg/kg only via *i.p.*

The crude extract managed by seven acute-subchronic days presented antagonistic muscarinic effect in the young and adult animals. The muscarinic receptors, mainly the subtype M1, seem to mediate the main behavioral effects associated to acetylcholine, that is, effects on learning, short term memory and reactivity (Rang, 2003). The animals showed themselves more

quiet/stopped, but in alert state of accentuated attention.

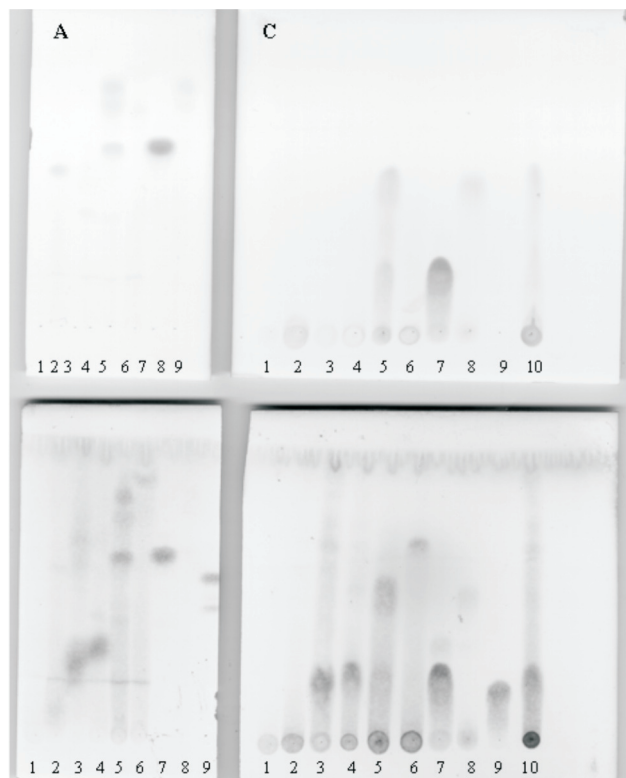
Izquierdo and colleagues (1999) reported that the cholinergic effect on the muscarinic receptors facilitate the working memory (known as immediate memory because it lasts from seconds to minutes); facilitate the memory formation of short (1 to 3 h) or long duration (3 to 8 h to consolidate). In the treatment of the adult animals it was investigated the cholinergic reversion made by the pre-training treatment - memory acquisition of short term. In the young animals it was investigated the cholinergic reversion made by the pre-test administration of the extract, allowing us to verify the evocation of the consolidated memory. As it addresses the evaluation of short and long term memory, it was possible to evaluate the effect of fraction SS administration, which being applied directly in the lateral ventricle, established connection with the other ventricles and regions related with memory and learning, demonstrated by the latency presented that *S. erecta* acts experimentally in the memory disturbances, with synergetic effect among the drugs used (crude extract and SS fraction of *S. erecta*; and scopolamine).

The present substances in the specie are of elevated molecular weight, due to the sugars linked to the flavonoid and saponin structures, and also by the polymers (tannins) that the extract contains (Goming et al, 2008). Saponins or saponosides form a heteroside group being this name due to the property that they have of forming abundant foam, as soap do, that emulsify the oil in water and that provides hemolytic effect (Dewick, 1997). But in the hematologic analysis from the treatment for seven days with 50 mg/kg, it was not seen neither hemolytic effect nor hemorrhage in the autopsy made.

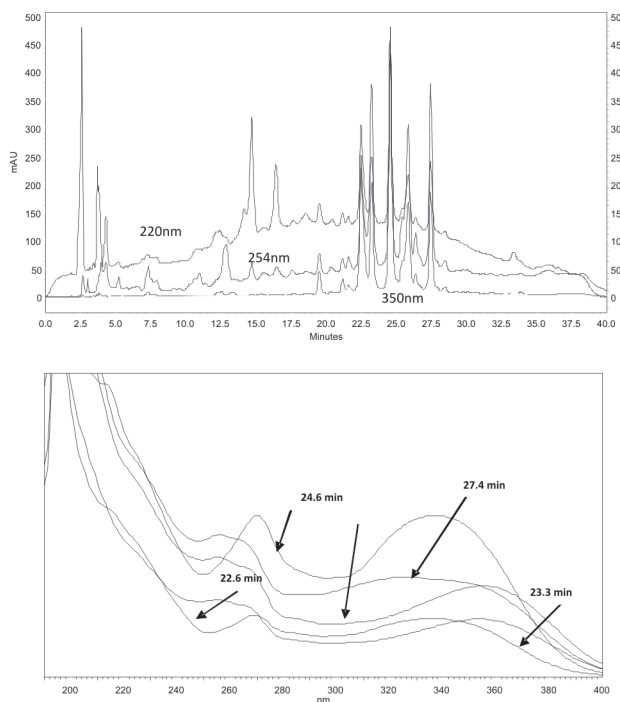
In the antioxidant evaluation, our results *in vitro* are passible to be applied *in vivo*, because the percentages were elevated and without (or low) toxicity detected in the other bioassays. These radicals are chemical species that have only one non-paired electron in an external orbital. In such state, the radical is extremely reactive and unstable, and takes part in reactions with inorganic chemical or organic substances, proteins, lipids, and carbohydrates, particularly with important molecules in the membranes and nucleic acids. Moreover, these reactive species initiate autocatalytic reactions where the molecules that react with are converted themselves in free radicals to propagate the damages series (Cotran et al., 1999). But the serum, the teciduals liquid and the cells possess antioxidant mechanisms of protection that annul this toxicity - ceruloplasmine, transferrine, superoxide dismutase, catalase and glutation peroxidase. Many of the protective effects in CNS are related to the presence of poliphenolic constituents of the vegetable extracts, being able to act in different forms, contributing thus for the neuronal tissue integrity inhibiting the activity of the enzyme superoxide dismutase (it acts as blocking of FR's propagator) and monoamine oxidase, that contribute to generate free radical (RL) in the brain and in the body;

kidnapping radical that could cause damage to the neurons and, consequently, retarding the changes associated with age in the brain; reducing the liberation of araquidonic acid, a toxic co-product to the lipidic metabolism, that appears on the brain as soon as the ischemic episode (Gold et al. 2002; Viegas Junior et al. 2004). Auddy et

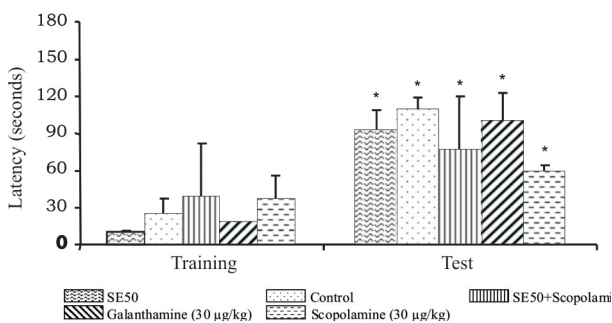
al. (2003) presented results with antioxidant plants of the Indian medicine: *Sida cordifolia*, *Cynodon dactylon* and *Evolvulus alsinoides*, that compose rasayanas in the ayurvedic practice for disturbances, neurodegenerations, prevention and treatment, as Parkinson and Alzheimer; as well as renewers, a lot in vogue in cosmetology as anti-



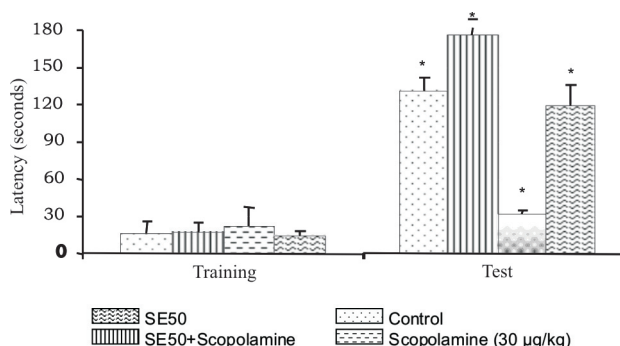
**Figure 1.** TLC profiles of *S. erecta* fractions. Mobile phase: BAW (*n*-butanol:acetic acid:water, 4:1:5; upper phase) (A and B) and  $\text{CHCl}_3$ :MeOH (4:1) (C and D). Detection reagents: NP/PEG (A and C) and vanillin-sulfuric acid (B and D). (1 to 6 = Sephadex LH20 fractions, 7 = rutin, 8 = flavonoid fraction, 9 = saponin fraction, 10 = aqueous extract).



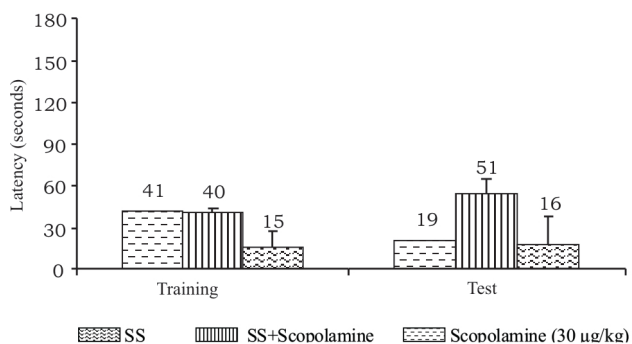
**Figure 2.** HPLC profile and spectra data of aqueous extract from *S. erecta*. HPLC equipment (Shimadzu®) using a reverse phase column (Supelcosil™ RP-18, 250 x 4.6 mm *i.d.*), monitored at 220, 254 and 350 nm. A two-solvent gradient system of water:acetic acid 0.1% (A) and MeOH (B) was used. The gradient program consisted of three periods: (1) 0-32 min, 10-66% B, (2) 32-35 min, 66-10% B, (3) 35-40 min, 10% B. The flow-rate was 1.0 mL/min.



**Figure 3.** Cognitive test in adult male Wistar rats (11 months) in the passive avoidance. SE50, crude extract of *S. erecta* dose 50 mg/kg. Control with distilled water, treatment seven days (acute/subchronic). Means±SD (n=10 for group). Comparison between training (acquisition) and test (retention) was compared [ $F(2,10) = 32.82$ ]  $p < 0.05$ , Tukey ( $r = 5$ ,  $x = 82.76$ ,  $dms = 22.75$ )  $p < 0.05$ .



**Figure 4.** Cognitive test in young male Wistar rats (3 months) in the passive avoidance. SE50, crude extract of *S. erecta* dose 50 mg/kg. Control with distilled water, treatment seven days (acute/subchronic). Means±SD (n = 10 for group). Comparison between training (acquisition) and test (retention) [ $F(2,8) = 10.29$ ]  $p < 0.05$ . Tukey ( $r = 4$ ,  $x = 114.50$ ,  $dms = 73.79$ )  $p < 0.05$ .



**Figure 5.** Cognitive test in adult male Wistar rats (6 months) in the passive avoidance with SS application (saponin fraction of *S. erecta* dose 5 mg/mL) in the right lateral ventricle (acute treatment). Means±SD ( $n = 5$  for group). Comparison between training (acquisition) and test (retention) [ $F(2,6) = 0.07$ ]. Tukey ( $r = 3, x = 32.28, dms = 38.60$ )  $p > 0.05$ .

aging. The oxidative evaluation consists in detecting new substances that can inhibit the harmful radical or to make potent the effects of those naturally produced *in vivo*.

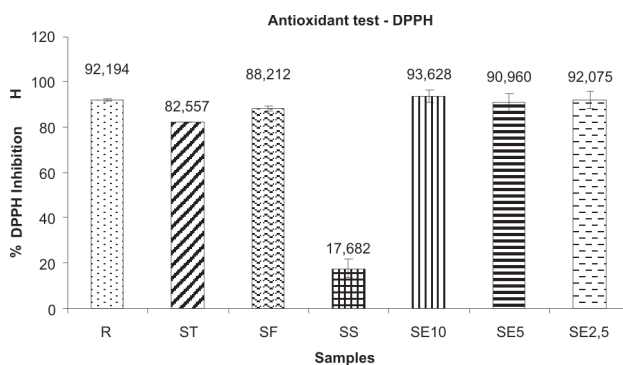
The glucose quantification in the blood reflects the point of momentary balance between its production, utilization and stock. Type I and II diabetes represents a group of metabolic disturbances in which the badly used glucose results in hyperglycemia. The control should be considered of how to keep adequated supplies of fuels (compulsory source for the brain) in the food ingestion and variable metabolic demands (*p.e.* exercise practice). The glycemia level is controlled by a feedback system between the liver, the muscle, the fat and the pancreatic isles, being the insulin the main regulator hormone between basal state (*p.e.* fast) and fed (Rang et al. 2003).

Triacylglycerides excess can increase LDL-cholesterol levels, and if not used for muscular work, they will contribute to coronary artery and cardiac mortality (Rang et al. 2003).

The determination of serum AST activity (aspartate aminotransferase) can be useful in hepatopathies, acute infarct of myocardium and myopathies. The increase of the enzymatic activity can be seen in mixedema, hemolytic anaemias and shock. Once the enzyme is also present in erythrocytes, the presence of hemolysis elevates the presence in the serum, what was not detected, annulling a possible activity by the presence of saponins in *S. erecta* crude extract, proved by the diagnosis of the red globules (morphology and normal quantification). ALT evaluation (alanine aminotransferase) is recommended for tracking of hepatitis, presenting no alterations in our diagnosis.

The urea evaluation can investigate pictures of renal inadequacy caused by toxic products and alteration in the metabolism. Exam of creatinine also is useful for the evaluation of the renal function (Al Chami et al. 2003).

The biochemical results demonstrate absence of toxicity and protective effects in the glucosidics evaluations, mainly by the great quantity of sugars in



**Figure 6.** Antioxidant evaluation. R: rutin (10 mg/mL); ST: tannin fraction (0.1 mg/200 µL); SF: flavonoid fraction (1.9 mg/500 µL); SS: saponin fraction (2.0 mg/500 µL); SE: crude extract *S. erecta* in the concentrations of 10 mg/mL, 5 mg/mL and 2.5 mg/mL.

the species. Once the acute-subchronic treatment did not present alterations in the renal rates, it indicates satisfactory glomerular filtration occurrence with low toxicity.

In the enzymatic evaluation, the fractions linked to the enzymes, demonstrating a larger inhibition on silica plate. This datum confirms the reversible activity of the cholinergic blocking due to the direct administration in CNS (ICV) or in the oral treatment. These results were stand-out since activity was found in the fractions and not in the crude extract, suggesting that the saponin and flavonoid fractions are able to cross the haematoencephalic barrier, making the pharmacological action in the central nervous system (CNS). Hilhouse et al. (2004) presented results in which flavonoid fractions of *Rhodiola rosea* were inhibitors of AChE as well as Savelev et al. (2004) in which terpenes of *Salvia* spp inhibited AChE and BuChE, demonstrating that other substances besides the alkaloids can inhibit cholinergic enzymes and improve the neurotransmission as bioactive antioxidant from *S. erecta*. Barbosa Filho et al. (2006) reviews plants and plant-derived compounds that inhibit enzyme acetylcholinesterase. This review refers to 309 plant extracts and 260 compounds isolated from plants.

## ACKNOWLEDGEMENTS

To the laboratory PROVET - Brazilian Institute of Diagnosis and Veterinary Specialties by the seriousness and excellence in the laboratorial diagnosis; Prof. Dr. José Roberto Giglio and Dra. Carolina B. A. Restini by English check contributions. To CNPq, CAPES and FAPESP for sponsorship.

## REFERENCES

- Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M 2003. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. *J Clin Pharm Ther* 28: 53-59.



- Al Chami L, Méndez R, Chataing B, O'Callaghan J, Usbillaga A, Lacruz L 2003. Toxicological effects of solamargine in experimental animals. *Phytother Res* 17: 254-258.
- Almeida RN, Falcão ACGM, Diniz RST, Quintans-Júnior LJ, Polari RN, Barbosa-Filho JM, Agra MF, Duarte JC, Ferreira CD, Antonioli AR, Araújo CC 1999. Metodologia para avaliação de plantas com atividade no sistema nervoso central e alguns dados experimentais. *Rev Bras Farm* 80: 72-76.
- Angelucci MEM, Vital MAB, Cesario C, Zadusky CR, Rosalen PL, Da Cunha C 1999. The effect of caffeine in animal models of learning and memory. *Eur J Pharmacol* 373: 135-140.
- Arruda APCCBN, Coelho RG, Honda NK, Ferrazoli C, Pott A, Hiruma-Lima CA 2009. Gastroprotective effect of *Serjania erecta* Radlk (Sapindaceae): involvement of sensory neurons, endogenous nonprotein sulfhydryls, and nitric oxide. *J Med Food* 12:1411-1415.
- Auddy H, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T, Mukherjee B 2003. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol* 84: 131-138.
- Barbosa Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde-Filho PF, Silva MS, da-Cunha EVL, Almeida JRGS, Quintans-Júnior LJ 2006. Natural products inhibitors of the enzyme acetylcholinesterase. *Rev Bras Farmacogn* 16: 258-285.
- Baranov A 1982. Medicinal use of ginseng and related plants in the Soviet Union: recent trends in the soviet literature. *J Ethnopharmacol* 6: 339-353.
- Brekhman II, Dardymov IV 1969. New substances of plant origin which increase non-specific resistance. *Ann Rev Pharmacol* 9: 419-432.
- Cacabelos R 2005. Pharmacogenomics and therapeutic prospects in Alzheimer's disease. *Expert Opin Pharmacother* 6: 1967-1987.
- Carlini EA 2003. Plants and the central nervous system. *Pharmacol Biochem Behav* 75: 504-512.
- Cerqueira ATAR, Oliveira NIL 2002. Programa de apoio a cuidadores: uma ação terapêutica e preventiva na atenção à saúde dos idosos. *Psicol USP* 13: 133-150.
- Cotran RS, Kumar V, Schoen FJ 1999. Robbins Pathologic Basis of Disease. 5<sup>th</sup> ed., W.B. Saunders Company, Philadelphia.
- Dahanukar SA, Thatte UM 1997. Current status of Ayurveda in phytomedicine. *Phytomedicine* 4: 359-368.
- Davydov MK, Krikorian AD 2000. *Eleutherococcus senticosus* (Rupt & Maxim) Maxim (Araliaceae) as an adaptogen: a closes look. *J Ethnopharmacol* 72: 345-393.
- Dewick PM 1997. Medicinal natural products: a biosynthetic approach. John Wiley & Sons, Chichester.
- Espínola EB, Dias RF, Mattei R, Carlini EA 1997. Pharmacological activity of guaraná (*Paullinia cupana* Mart.) in laboratory animals. *J Ethnopharmacol* 55: 223-229.
- Ferruck MS, Acevedo-Rodríguez P 2005. Três espécies novas de *Serjania* (Sapindaceae) para sudamérica. *Systematic Botany* 30: 153-162.
- Forlenza OV, Gattaz WF 1998. Influência de mecanismos colinérgicos nos processos neurodegenerativos relacionados à formação de amiloide e a fosforilação da proteína TAU. *Rev Psiq Clin* 25: 114-117.
- Girolami U, Anthony DC, Frosch MP, 1996. O Sistema Nervoso Central. In: Robbins SL, Cotran RS, Kumar V, Schoen FJ. Patologia Estrutural e Funcional. 5<sup>th</sup> ed. Rio de Janeiro: Guanabara-Koogan.
- Gold PE, Cahill L, Wenk GL 2002. Ginkgo biloba: a cognitive enhancer? *Psychol Sci Publ Int* 3: 2-9.
- Gomig F, Pietrovski EF, Guedes A, Dalmarco EM, Calderari MT, Guimarães CL, Pinheiro RM, Cabrini DA, Otuki MF 2008. Topical anti-inflammatory activity of *Serjania erecta* Radlk (Sapindaceae) extracts. *J Ethnopharmacol* 118: 220-224.
- Guarim Neto G, Santana SR, Silva JVB 2000. Notas etnobotânicas de espécies de Sapindaceae Jussieu. *Acta Bot Bras* 14: 327-334.
- Halks-Miller M, Schroeder BS, Haroutunian V, Moening U, Rossi M, Achim C, Purohit D, Mahmoudi M, Horuk R 2003. CCR1 is an early and specific marker of Alzheimer's disease. *Ann Neurol* 54: 638-646.
- Hilhouse BJ, Ming DS, French CJ, Towers GHN 2004. Acetylcholine esterase inhibitors in *Rhodiola rosea*. *Pharm Biol* 42: 68-72.
- Howes MR, Houghton PJ 2003. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol Biochem Behav* 75: 513-527.
- Izquierdo I, Medina JH, Vianna MRM, Izquierdo LA, Barros DM 1999. Separate mechanisms for short- and long-term memory. *Behav Brain Res* 103: 1-11.
- Jordan-Sciuotto KL, Malaiyandi LM, Bowser R 2002. Altered distribution of cell cycle transcriptional regulators during Alzheimer disease. *J Neuropathol Exp Neurol* 61: 358-367.
- Kidd PM 1999. A review of nutrients and botanicals in the integrative management of cognitive dysfunction. *Altern Med Rev* 4: 144-161.
- Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal* 13: 8-17.
- Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, Tu MT 2005. Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinol* 30: 225-242.
- Marston A, Kissling J, Hostettmann KA 2002. A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochem Anal* 13: 51-54.
- Mattei R, Dias RF, Espínola EB, Carlini EA, Barros SBM 1998. Guaraná (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity in vitro. *J Ethnopharmacol* 60: 111-116.
- Mori M, Hasegawa N 2003. Superoxide dismutase activity enhanced by green tea inhibits lipid accumulation in 3T3-L1 cells. *Phytother Res* 17: 566-567.
- Panossian A, Gabrielian E, Wagner H 1997. Plants adaptogens: II. Bryonia as an adaptogen. *Phytomedicine* 4: 85-99.
- Panossian A, Wikman G, Wagner H 1999. Plants adaptogens: III. Earlier and more recent aspects and concepts on their mode of action. *Phytomedicine* 6: 287-300.
- Paxinos G, Watson C 1998. The rat brain in stereotaxic coordinates. 4<sup>th</sup> ed., Academic Press.
- Perry EK, Pickering AT, Wang WW, Houghton P, Perry NS 1998. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *J Altern Complement Med* 4: 419-428.
- Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NSL 1999. Medicinal plants and Alzheimer's disease: integrating ethnobotanical and contemporary scientific evidence. *J Pharm Pharmacol* 51: 527-534.

- Pott A, Pott VJ, Sobrinho AB 2004. Plantas úteis à sobrevivência no Pantanal. EMBRAPA, Corumbá, Brazil, p. 5.
- Rai D, Bhatia G, Palit G, Pal R, Singh S, Singh HK 2003. Adaptogenic effect of *Bacopa monniera* (Brahmi). *Pharmacol Biochem Behav* 75: 823-830.
- Rang HP, Dale MM, Ritter JM, Moore PK 2003. Pharmacology. 5<sup>th</sup>ed., Churchill Livingstone, Edinburgh.
- Robbins SL, Cotran RS, Kumar V, Schoen FJ 1999. Robbins Pathologic Basis of Disease. 5th ed., W.B. Saunders Company, Philadelphia.
- Savelev SU, Okello EJ, Perry EK 2004. Butyryl- and acetyl-cholinesterase inhibitory activities in essential oils of *Salvia* species and their constituents. *Phytother Res* 18: 315-324.
- Singh B, Chandan BK, Gupta DK 2003. Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun. (part II). *Phytother Res* 7: 531-536.
- Siripuraru KB, Gupta P, Bhatia G, Maurya R, Nath C, Palit G 2005. Adaptogenic and anti-amnesic properties of *Evolvulus alsinoides* in rodents. *Pharmacol Biochem Behav* 81: 424-432.
- Stahl SM 1996. Essential psychopharmacology: neuroscientific basis and practical applications. Cambridge University Press, New York.
- Viegas Junior C, Bolzani VS, Furlan M 2004. Produtos naturais como candidatos a fármacos úteis no tratamento do mal de Alzheimer. *Quim Nova* 27: 665-660.