SaBios
Revista de Saúde e Biologia

ARTIGOS COMPLETOS/COMPLET ARTICLES

ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF ETHANOL EXTRACTS FROM FOUR *PIPER* SPECIES

EFEITOS ANTIDEPRESSIVOS E ANSIOLÍTICOS DO EXTRATO ETANÓLICO DE QUATRO ESPÉCIES DE *PIPER*

Silvia Aparecida Oesterreich[(1)], Giseli Karenina Traesel[(1*)], Ana Claudia Piccinelli[(1)], Diana Figueiredo Santana Aquino[(1)], Cândida Aparecida Leite Kassuya[(1)], Jonas Mota (2), Celio Estanislau(3)

[1]Universidade Federal da Grande Dourados, Faculdade de Ciências da Saúde, Dourados, Mato Grosso do Sul, Brasil. [2]Universidade Estadual do Mato Grosso do Sul, Departamento de Química, Dourados, Mato Grosso do Sul, Brasil.[3]Universidade Estadual de Londrina, Departamento de Psicobiologia, Londrina, Brasil.
Endereço para correspondência: Faculdade de Ciências da Saúde, Rodovia Dourados - Itahum KM 12, PO Box – 322, 79800-000 – Dourados-MS, + 55 67 3410-2665, giselitraesel@gmail.com

ABSTRACT

This study objected to assess the pharmacological effects of ethanol extracts from *Piper aduncum* L. (AD), *Piper glabratum* (GL) Kunth, *Piper amalago* (AM) and *Piper visosanum* Yuncher (VIS) on the CNS in animal models. Therefore, we used a quantitative type research, where the rats were allocated into six experimental groups AD, GL, VIS, AM, NC (negative control) and PC (positive control). Were performed the elevated plus-maze, open-field and forced swimming tests. The extracts were orally administered (300 mg/kg) diary single dose, thirty minutes before testing. A significant increase was observed in locomotors activity and the time spent on the open arms in the AD group, when compared to the NC group, suggesting an anxiolytic effect. VIS group also exhibited a potential anxiolytic effect, since exploratory activity increased significantly relative to the PC group in the open field test. GL, in the forced swim test underwent a significant reduction in the time and frequency of swimming compared to the PC group, which decreases in active behavior, indicating a possible depressive effect. AD demonstrated antidepressant effects in the forced swimming test when compared to the PC. AM significantly increased the activity of self-cleaning when compared to the NC group, reinforcing the possible anxiogenic effect of the species.
Key Words: *animal models; anxiety; depression; Piper spp.*

RESUMO

Este estudo teve por objetivo avaliar os efeitos farmacológicos do extrato etanólico de *Piper aduncum* L. (AD), *Piper glabratum* (GL) Kunth, *Piper amalago* (AM) e *Piper visosanum* Yuncher (VIS) no SNC em modelos animais. Para tanto, utilizou-se de uma pesquisa tipo quantitativa, aonde os ratos foram alocados em seis grupos experimentais AD, GL, AM, VIS, NC (controle negativo) e PC (controle positivo). Foram realizados os testes de labirinto em cruz elevado, campo aberto e natação forçada. Os extratos foram administrados via oral (300 mg/kg) em uma única dose diária, 30 minutos antes dos testes. Um aumento significativo foi observado na atividade locomotora e o tempo gasto nos braços abertos no grupo AD, em comparação com o grupo NC, sugerindo efeito ansiolítico. O grupo VIS também exibiu potencial efeito ansiolítico, uma vez que a atividade exploratória aumentou significativamente em relação ao grupo PC no teste do campo aberto. GL, no teste de natação forçada, apresentou redução significativa no tempo e frequência de nado em comparação do grupo PC, que diminuiu seu comportamento ativo, indicando possível efeito depressivo. AD demonstrou efeito antidepressivo no teste de natação forçada quando comparado ao PC. AM aumentou significativamente a atividade de auto limpeza, quando comparado ao grupo NC, reforçando o possível efeito ansiogênico da espécie.
Palavras-Chave: *modelos animais; ansiedade; depressão; Piper spp.*

INTRODUCTION

The genus *Piper* belongs to the Piperaceae family, which has more than 700 species. These species are commercially, economically and medicinally significant. Plants from the genus *Piper* have been used for several purposes, including many traditional medicinal practices, which are widely distributed in tropical and subtropical regions of the world (1,2).

By analyzing the species of *P. glabratum* and *P. acutifolium*, nine new benzoic acids were found in their composition, four of which were previously known (3). In the toxicological assessment of the essential oil of *P. aduncum* L., Sousa and col. (4), concluded that a dose of LD50 2.400 ± 191.7 mg / kg has a wide margin of safety, with minimal toxic effects on hematological and biochemical parameters. Dillapiole is its main constituent (45.9%). It was shown to be efficient against fungal strains and exhibited some analgesic, anti-thrombotic and (limited) anti-platelet action. Extremely limited antioxidant and antimutagenic properties were also observed (4). It can also be used for treating neurological/mental disorders (5). Similarly, it was reported to exert an anticonvulsant effect (6) and exhibited moderate activity on the GABAA-benzodiazepine receptor (7).

Anxiety disorders are among the most common types of mental illness currently observed in the world and, thus, researches on their pharmacological treatment are of great interest (4). Benzodiazepines have been misused in the last 45 years by being considered the first choice of drugs used in the treatment of various forms of anxiety (8). Although providing many benefits, benzodiazepines also have several adverse effects including muscle relaxation, anterograde amnesia, addiction and sedation (9).

By using the benzodiazepines approximately 43% of patients have anxiety disorders, as a form of side effect, and end up eventually seeking some form complementary therapies (10). In this context, many pharmaceutical companies try to find alternative medicines with less adverse effects and more specific anxiolytic effects.

The aim of the present study was to analyze the pharmacological effects of the species *Piper aduncum* L., *Piper glabratum* Kunth, *Piper amalago* and *Piper visosanum* Yuncher on behavior, anxiety and depression, using rats as an experimental model, because, with the exception of *P. amalago*, there are no existing studies about the central actions of these substances in the literature.

MATERIAL AND METHODS

Plant Material

Piper amalago, P. aduncum, P. glabatum and P. vicosanum leaves were collected in Dourados – MS, in August 2008, and identified by Dr Elsie Franklin Guimarães (*Instituto de Pesquisas Jardim Botânico do Rio de Janeiro*, RJ, Brazil). The specimens, *P. amalago* (DDMS 4410), *P. aduncum* (DDMS 4413), *P. glabatum* (DDMS 4412) and *P. vicosanum* (DDMS 4411), were deposited in the herbarium of the *Universidade Federal da Grande Dourados, Mato Grosso do Sul*, Brazil. The species were collected at the following geographical coordinates: *P. amalago* (S22^{0}12'42.9", WO54^{0}54'55.6"); *P. aduncum* (S22^{0}12'42.9", WO54^{0}54'55.5"); *P. glabatum* (S22^{0}12'37.7", WO54^{0}55'03.2") and *P. vicosanum* (S22^{0}12'37.8", WO54^{0}55'02.6").

Preparation of the ethanol extract

The leaves of *P. amalago* (816 g), *P. aduncun* (900 g), *P. glabatum* (850 g) and *P. vicosanum* (1100 g) were dried at room temperature and ground into powder. They were then submitted to extraction by maceration with ethanol 92% (3 x 2000 ml) for 7 days. The extract was concentrated on a rotary evaporator, under reduced pressure, and then dried, with a yield of 0.94%, 4.2%, 3.6% and 4.8%, respectively.

Phytochemical screening

Tests were performed to identify the following classes of substances: alkaloids, steroids/triterpenoids, flavonoids, organic acids, phenols and tannins (11).

Phytochemical analysis of ethanol extract of *Piper amalago*

The obtainment and chemical composition of *P. amalago* ethanolic extract (30) used in this study, was previously

described by our group with the same quantity of vegetal drugs (12).

Animals

Male Wistar rats (2-3 months old, weighing between 150-250 g) were randomly allocated into six groups of five animals each. All animals were kept in a controlled temperature (21°C ± 2°C), humidity (60%) and luminosity (12h/12h), with a diet of water and food *ad libitum.* This experiment was approved by the Ethical Committee for Animal Experiments of the *Universidade Federal da Grande Dourados* under protocol number 011/2012. All procedures were performed according to the Brazilian College of Animal Experimentation, always aiming for the lowest possible levels of suffering for the animals.

Drugs and pharmacological procedures

The six experimental groups were as follows: *Piper amalago* (AM); *P. aduncum* (AD); *P. glabatum* (GL); *P. vicosanum* (VIS); a negative control group (NC) and a positive control group (PC). The extracts were orally administered at a dosage of 300 mg/kg in saline solution. The dose levels of the extracts were selected based on the information obtained from studies previously described by our group (12).

The NC group received only saline solution (vehicle), whereas the PC group received benzodiazepine Diazepam (Valium®, Roche), administered by intraperitoneal injection at a dosage of 1 mg/kg. Diazepam has been used as a reference compound in behavioral studies (13). The administration was performed once a day, thirty minutes before testing. The open field test was performed on the first day. The elevated plus-maze test and the forced swimming test were conducted on the second day. The tests were performed in a sound-proof, brightly illuminated room between 08:00 and 11:00 h.

Open Field Test

The animals were exposed to an open field (40 cm x 50 cm x 60 cm) with a white floor divided into 12 identical squares and delimited by black lines. Each animal was placed in the center of the arena and its behavior was analyzed for 3 minutes. Ambulation, rearing, grooming and latency time were counted and used as locomotion and exploration measures (14).

Elevated Plus-Maze Test

The apparatus consisted of two open arms (50 cm x 10 cm) and two closed arms (50 cm x 10 cm x 40 cm), arranged such that the two arms of each type were opposite to each other. Animals were placed individually in the central area of the maze (equal choice of entering an open or closed arm) and observed for 3 minutes. The latency time to enter one of the arms was analyzed, as was the number of entries and the time spent in the open and closed arms (14).

Forced Swimming Test

The protocol was performed in a cylindrical container of 21 cm height and 20.5 cm in diameter, with a water column of 13 cm, at a temperature of 23 ° C (± 2 ° C). One day before the test, the rats were individually placed in the water for 15 minutes. In the next day, the same procedure was repeated, but session duration was limited to 5 minutes. These sessions were video-recorded for later analysis. The behavior exhibited was classified as frequency of swimming, swimming time, immobility times (15), after analysis with the X-Plo-Rat software (16).

Statistical analysis

The data are presented as mean ± S.E.M. The differences between groups were assessed by analysis of variance (one-way ANOVA), followed by the Newman-Keuls test. Statistical differences were considered to be significant at $p < 0.05$.

RESULTS

The results of the phytochemical screening of the leaves of *P. aduncum L.*, *P. glabratum* Kunth, *P. amalago* and *P. visosanum* Yuncher are displayed in Table 1. For the species *P. vicosanum* Yuncher, the tests indicated the presence of alkaloids, tannins, steroids and triterpenoids. For the species *P. aduncum*, the tests indicated the

presence of organic acids, tannins, flavonoids, steroids and triterpenoids. The predominance of steroids and triterpenoids for this species has previously been described in other studies (2). The tests indicated that *P. glabratum* has the following classes of compounds: organic acids, tannins, steroids and triterpenoids. Previous studies of *P. glabratum* resulted in the isolation of benzoic acid derivatives (3). For *P. amalago*, the tests indicated the presence of alkaloids, organic acids, tannins, steroids and triterpenoids. Previous studies of this species have been demonstrated an accumulation of amides (class detected in the chemical test) pyrrolidines (17-20).

Table 1 - Results of phytochemical screening with reagents

Test	*P. vicosanum*	*P. aduncum*	*P. glabratum*	*P. amalago*
Alkaloids (Dragendorff Reagent)	+++	+	-	+++
Alkaloids (Mayer`s Reagent)	+++	+	-	+++
Alkaloids (Bouchardat Reagent)	+++	+	-	+++
Organic Acids	-	+	+++	+
Phenols and Tannins	+	+	+++	+
Flavonoids	-	+	-	-
Steroids and Triterpenoids	+	++	++	++

+++large presence, ++ remarkable presence, + slight presence, - absence

The chromatographic fractionation of the ethanol extract of *P. amalago* conducted by our research group, resulted in the isolation amides pyrrolidines and piperidines (12). The mass of the amides isolated was insufficient for testing.

In the elevated plus-maze test (Table 2), the group that received *P. aduncum* extracts (mean ± S.E.M = 124.9 ± 41.3, $p < 0.05$) showed increased the time spent in the open arms and increased locomotors activity (mean ± S.E.M = 50.0 ± 24.3, $p < 0.05$), when compared to the NC (1 mL/kg; mean ± S.E.M = 0.8 ± 1.8, $p < 0.05$) and the PC group (1 mg/kg; mean ± S.E.M = 2.3 ± 5.3, $p < 0.05$), thereby suggesting an anxiolytic effect.

Table 2 - Results of Elevated Plus-maze Test

Elevated Plus-maze Test (Mean ± SD)	
Group	**Time in Open Arm (s)**
P. amalago	0.6698 ±1.4977
P. glabratum	24.1294 ±26.1394
P. vicosanum	5.8962 ±8.4989
P. aduncum	124.9762[a,b] ±41.3895
Positive Control	2.3776 ±5.3165
Negative Control	0.8452 ±1.8899

a= significant differences when compared to the positive control (*P*<0.05)
b= significant differences when compared to the negative control (*P*<0.05)

In the forced swimming test (Table 3), the *P. glabratum* extract decreased considerably active behavior, such as the frequency (mean ± S.E.M = 8.2 ± 1.4, p < 0.05), and the swimming time (mean ± S.E.M = 1.7 ± 0.3, p < 0.05), when compared to NC frequency (mean ± S.E.M = 14.4 ± 2.3, p < 0.05) and time (mean ± S.E.M = 3.0 ± 0.4, p < 0.05), and when compared to the PC group frequency (1mg/kg; mean ± S.E.M = 16.2 ± 3.5, p < 0.05) and time (1mg/kg; mean ± S.E.M = 3.7 ± 0.8, p < 0.05), indicating a possible depressive effect.

Table 3 - Results of Forced Swimming Test

Forced Swimming Test (Mean ± SD)			
Group	**Frequency of Swimming**	**Swimming time (s)**	**Immobility times (s)**
P. amalago	11.4 ± 2.51	2.7966 ± 0.7828	12.6 ± 3.362
P. glabratum	8.2[a,b] ± 1.483	1.7236[a,b] ± 0.3123	12.2 ± 5.263
P. vicosanum	11.6 ± 3.362	2.5606 ± 0.8559	13.4 ± 1.673
P. aduncum	9.8 ± 1.789	2.8024 ± 0.4705	6.6[a] ± 2.51
Positive Control	16.2 ± 3.564	3.7392 ± 0.8487	14.2 ± 2.28
Negative Control	14.4 ± 2.302	3.0382 ± 0.4548	11.4 ± 3.362

a= significant differences when compared to the positive control (*P*>0.05)
b= significant differences when compared to the negative control (*P*>0.05)

Ethanolic extract from *P. aduncum* demonstrated antidepressant effects in the forced swimming test since it decreased significantly the mobility in relation to the PC group (*P*= 0.01).

In the Open Field Test *P.glabratum* increased significantly the locomotors activity (300mg/kg; mean±S.E.M=4,0±1,4, p<0,05) compared to NC (300mg/kg; mean±S.E.M=5,0±3,6, p<0,05) and diazepam (1mg/kg; mean±S.E.M=2,2±1,3, p<0,05), indicating anxious behavior response and

anxiogenic effect. The same effect was observed in *P. amalago,* significantly decrease in exploratory activities of animals (300mg/kg; mean±S.E.M=0,4±0,5, p<0,05) compared to NC (300mg/kg; mean±S.E.M=3,8±1,3, p<0,05). The extract of this specie increased significantly the self cleaning activities (300mg/kg; mean±S.E.M=4,6±1,8, p<0,05) compared to NC (300mg/kg; mean±S.E.M=1,2±1,0, p<0,05) and diazepam (1mg/kg; mean±S.E.M=0,8±1,3, p<0,05), reinforcing the possible anxiogenic potential of this plant. These data to corroborate the results obtained by Lopes et. Al. (2012). Otherwise *P.vicosanum* presented potential anxiogenic effect because increased significantly the exploratory activities of animals (300mg/kg; mean±S.E.M=3,2±1,4, p<0,05) compared to diazepam group (1mg/kg; mean±S.E.M=0,8±0,8, p<0,05).

In the open field test, the *P. glabratum* extract increased significantly the locomotors activity, represented by crossings number when compared to NC (*P*=0.01) and the PC group (*P*=0.03), indicating an anxiogenic effect. *P. amalago* significantly increased the activity of grooming when compared to NC (*P*=0.00) and the PC group (*P*=0.00), reinforcing the possible anxiogenic potential of the species.

It is conceded that rearing is a function of the excitability level of the CNS (21,22). *P. vicosanum* exhibited a potential anxiolytic effect, since it increased significantly exploratory activity when compared to the PC group (*P*=0.04).

DISCUSSION

The present study investigated the effects of ethanol extracts from *P. aduncum* L., *P. glabratum* Kunth, *P. amalago* and *P. visosanum* Yuncher on the central nervous system (CNS). With the exception of *P. amalago*, there are no studies about the central actions of these substances in the literature. The presence of phenolic compounds, alkaloids, essential oils, flavonoids, tannins and amides, in some species from the genus *Piper,* may justify the neurobehavioral responses found in this study.

Flavonoids and essential oils are the main chemical classes with CNS depressant and anxiolytic effects, but alkaloids also present similar effects. In many cases, the alkaloids interact directly with molecular targets within the nervous system (23). For instance, individual alkaloids act as agonists and antagonists to a variety of neurotransmitter systems through direct binding to neuroreceptors interfering with neurotransmitter metabolism (e.g. cholinesterase inhibition), signal transduction, and ion channel function (23), or by mimicking the structure of endogenous neurochemicals (24). Evidence suggests that extracts with terpene or phenolic actives owe their effects to multifarious synergies between their component chemicals (25,26). This factor, along with an inability to reliably standardize extract constituents, it has to date constrained their development and the clarity of the literature on their efficacy in humans.

Phenolics are ubiquitously found across the plant kingdom, with ~10,000 structures identified to date. With a few notable exceptions, phenolic compounds are synthesized from precursors produced by the phenylpropanoid pathway. Structurally, they share at least one aromatic hydrocarbon ring with one or more hydroxyl groups attached. The simplest compound with this structural motifis the phenol molecule, which itself does not occur in plants. Phenolics range from simple low-molecular weight compounds, such as the simple phenylpropanoids, coumarins, and benzoic acid derivatives, to more complex structures such as flavanoids, stilbenes, and tannins. In terms of CNS function, a wide range of phenolic compounds interact directly with neurotransmitter systems (27). The phenolic compounds, particularly those like flavonoids, which are ubiquitously consumed in plant-based foods, may then owe the balance of their CNS effects to the latter (but with notable exceptions in terms of hormonal effects and GABAergic effects).

Anxiety is a symptom that accompanies various CNS disorders, and as a disorder by itself, it is characterized by a tense and exhaustive physical alertness in humans (28). Ethnomedical and pharmacological knowledge about the genus *Piper* would allow us to presume that they engage in

anxiolytic activity in the CNS, which could be oriented to decrease anxiety in patients.

The effects on locomotors and exploratory activities may be due to the presence of amides in some species of the genus *Piper,* which affect the CNS. Piplartine has antidepressant and anxiolytic effects when administered to rats (29). Pan and col. (30) investigated the constituent responsible for the antidepressant action of *P. laetispicum*, observed in the forced swimming test in mice, and identified three amides. Compounds isolated from Piper, such as *kapavironas* and *piperinas,* have exhibited depressant activities in the CNS, as well as sedative and anticonvulsant effects, diminished reflexes (5) and insomnia. The substance α-pyrones (*kavapironas*) has been identified as responsible for CNS activity (31).

In this study, the forced swim test the dose of *P. glabratum* significantly decreased the frequency and duration of swimming, indicating CNS depressant response. In the open field test a statistically significant decrease was observed in time and frequency of rearing, affecting the exploratory ability of the animal. Also decreases ambulation, indicating anxiogenic behavior. There were no significant differences in other behaviors analyzed.

The *P. vicosanum* ethanol extract significantly increased the frequency of rearing during the open field test compared to the PC group, indicating a possible anxiolytic effect. In the elevated plus-maze test the group that received *P. aduncum* extracts increased considerably the permanent time of open arms and increased locomotor activity, when compared to the NC and the PC group, thereby suggesting an anxiolytic effect. The *P. aduncum* ethanol extract was classified as having a low acute toxic effect on female rats (32), without evidence of behavioral or anatomic modification. The *in vitro* genotoxic effect, assessed by single cell gel electrophoresis (comet assay), on bone marrow cells was negative. The *in vivo* mutagenicity assessment of the micronucleus of bone marrow cells, as well as the *in vitro S. cerevisiae* strain mutation, confirmed the low mutagenicity.

CONCLUSION

It may be concluded that the ethanol extract derived from *P. aduncum* and *P. vicosanum* could be considered as a potential alternative for possible application in the pharmaceutical industry to prevent anxiety and depression. Further studies are necessary to elucidate anti-anxiety mechanisms and their potential clinical use in terms of treatment of anxiety and depression.

REFERENCES

(1) POTZERNHEIM, M.C.L.; BIZZO, H.R.; VIEIRA, R.F. Análise dos óleos essenciais de três espécies de Piper coletadas na região do Distrito Federal (Cerrado) e comparação com óleos de plantas procedentes da região de Paraty, RJ (Mata Atlântica). **Revista Brasileira de Farmacognosia**, v. 16, p. 246-251, 2006.

(2) PARMAR, V.S.; et al. Phytochemistry of the genus Piper. **Phytochemistry**, v. 46, p. 597-673, 1997.

(3) FLORES, N.; et al. Benzoic acid derivatives from Piper species and their antiparasitic activity. **Journal of Natural Products**, v. 71, p. 1538-1543, 2008.

(4) SOUSA, F.C.; et al. Plantas medicinais e seus constituintes bioativos: Uma revisão da bioatividade e potenciais benefícios nos distúrbios da ansiedade em modelos animais. **Revista Brasileira de Farmacognosia**, v. 18, p. 642-54, 2008.

(5) BOURBONNAS-SPEAR, N.; et al. Plant use by the Q'Eqchi'Maya of Belize in ethnopsychiatry and neurological pathology. **Society for Economic Botany**, v. 59, p. 326-36, 2005.

(6) NSOUR, W.; LAU, C.; WONG, I. Review on phytotherapy in epilepsy. **Seizure**, v. 9, p. 96-107, 2000.
(7) STAFFORD, G.I.; JÄGER, A.K.; VAN STADEN, J. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. J. **Ethnopharmacology**, v. 100, p. 210-215, 2005.
(8) RABBANI, M.; SAJJADI, S.; JALALI, A. Hydroalcohol extract and fractions of Stachys *lavandulifolia Vahl*: effects on spontaneous motor activity and elevated plus-maze behavior. **Phytotherapy Research**, v. 19, p. 854-858, 2005.
(9) TRIKHA, A.; REWARI, V. Sedation, analgesia and muscle relaxation in the intensive care unit. **Indian Journal of Anaesthesia**, v. 52, p. 620-631, 2008.
(10) ERNST, E. Herbal remedies for anxiety? A systematic review of controlled clinical trials. **Phytomedicine**, v. 13, p. 205-208, 2006.
(11) WALL, M.E.; et al. Steroidal sapogenins. XII. Survey of plants for steroidal sapogenins and other constituents. **Journal of American Pharmacists Association**, v. 43, p. 503-505, 1954.
(12) NOVAES, A.S.; et al. Diuretic and antilithiasic activities of ethanolic extract from *Piper amalago* (Piperaceae). **Phytomedicine**, v. 21, p. 523-528, 2013.
(13) REX, A.; MORGENSTERN, E.; FINK, H. Anxiolytic-like effects of Kava-Kava in the elevated plus maze test—a comparison with diazepam. **Progress in Neuro-psychopharmacology & Biological Psychiatry**, v. 26, p. 855-860, 2002.
(14) VIANA, C.C.S.; et al. Gamma-decanolactone effect on behavioral and genotoxic parameters. **Life Sciences**, v. 80, p. 1014-1019, 2007.
(15) CRYAN, J.F.; MARKOU, A.; LUCK, I. Assessing antidepressant activity in rodents: recent developments and future needs. **Trends in Pharmacological Sciences**, v. 23, p. 238-245, 2002.
(16) CHAIM, K.T.; MORATO, S. "X-Plot-Rat [Programa FREEWARE para registro comportamental]. Available in: http://scotty.ffclrp.usp.br/download.php?view.2, accessed in November 2013.
(17) ACHENBACH, H.; FIETZ, W.; WÔRTH, J.; WAIBEL, R.; PORTECOP, J. Investigations of the constituents of *Piper amalago* -30 new amides of the Piperine-Type. **Planta Medica**, v. 52, p. 12-18, 1985.
(18) DOMINGUEZ, X.; VERDE, J.; SUCAR, S.; TREVIÑO, R. Two amides from *Piper amalago*. **Phytochemistry**, v. 25, p. 239-240, 1985.
(19) JACOBS, H.; et al. Amides of *Piper amalago* var. nigrinodum. **Journal of the Indian Chemical Society**, v. 76, p. 713-717, 1999.
(20) CARRARA, V.S.; et al. HPLC analysis of supercritical carbon dioxide and compressed propane extracts from *Piper amalago* L. with antileishmanial activity. **Molecules**, v. 17, p. 15-33, 2011.
(21) LOPES, J.J.; et al. Neurobehavioral and toxicological activities of two potentially CNS-acting medicinal plants of Piper genus. **Experimental and Toxicologic Pathology**, v. 64, p. 9-14, 2012.
(22) SANTOS, F.B.; et al. Chemical composition and anxiolytic-like effects of the *Bauhinia platypetala.* **Revista Brasileira de Farmacognosia**, v. 22, p. 507-516, 2012.
(23) WINK, M. Interference of alkaloids with neuroreceptors and ion channels. **Studies in Natural Products Chemistry**, v. 21, p. 3–122, 2000.
(24) WINK, M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. **Phytochemistry**, v. 64, p. 3–19, 2003.
(25) WILLIAMSON, E.M. Synergy and other interactions in phytomedicines. **Phytomedicine**, v. 8, p. 401–409, 2001.
(26) SPINELLA, M. The importance of pharmacological synergy in psychoactive herbal medicines. **Alternative Medicine Review**, v. 7, p. 130–137, 2002.
(27) KENNEDY, D.O.; WIGHTMAN, E.L. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. **Advances in Nutrition**, v. 2, p. 32-50, 2011.
(28) JACKSON, M.; TURKINGTON, D. Depression and anxiety in epilepsy. **Journal of Neurology, Neurosurgery and Psychiatry**, v. 76, p. 45-47, 2005.
(29) SOUZA, L.O.; SILVEIRA, A., SILVEIRA, E.R.; VIANA, G.B. Piplartine, an amide alkaloid from *Piper tuberculatum*, presents anxiolytic and antidepressant effects in mice. **Phytomedicine**, v. 14, p. 605-612, 2007.
(30) PAN, S.; et al.. Antidepressant amides from *Piper laetispicum C.* **Acta**

Pharmacologica Sinica, v. 40, p. 355-357, 2005.
(31) MESQUITA, J.; et al. Estudo comparativo dos óleos voláteis de algumas espécies de Piperaceae. **Revista Brasileira de Farmacognosia**, v. 15, p. 6-12, 2005.
(32) SANTIN, J.; et al. Evaluation of the acute toxicity, genotoxicity and mutagenicity of ethanol extract of *Piper aduncum*. **Journal of Medicinal Plants Research**, v. 5, p. 4475-4480, 2011.

Enviado: 18/05/2014
Aceito: 20/11/2014
Publicado: 10/02/2014