Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **32** (8): 1224-30 (2013)

Regular Article
Received: December 13, 2012
Revised version: August 7, 2013
Accepted: August 11, 2013

Valepotriate Hydrines Isolated from an Anticonvulsant Fraction of *Valeriana pavonii* Poepp. & Endl.

Sara E. GIRALDO [1]*, Javier RINCÓN [1], Mario F. GUERRERO [1], Isabel LÓPEZ [2], Ignasio A. JIMÉNEZ [2], Mariel MARDER [3], Cristina WASOWSKI [3] & Nadezdha E. VERGEL [1]

[1] *Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, A.A. 14490, Bogotá, D.C, Colombia. 571-3165000 ext. 14662*
[2] *Instituto Universitario de Bio-Orgánca "Antonio González", Universidad de La Laguna, Avda. Astrofísico. Francisco Sánchez 2, La Laguna, 38206, Tenerife, Islas Canarias, España*
[3] *Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 (1113), Buenos Aires, Argentina*

SUMMARY. The present study deals with the isolation and identification of three valepotriate hydrines that are first reported in *Valeriana pavonii* Poepp. & Endl. (Valerianaceae), which were obtained from a dichloromethane fraction showing anticonvulsant activity *in vivo*. The isolation and purification of dichloromethane fraction was carried out by chromatographic techniques. The compounds were identified by comparison of their ^{1}H and ^{13}C NMR spectra with previously published data in scientific literature. Maximal electroshock seizure was used as *in vivo* pharmacological test, additionally *in vitro* GABA-A/BDZ-binding site studies were performed. Three valepotriate hydrines: valtrate acetoxyhydrine (**1**), valtrate isovaleroyloxyhydrine (**2**) and valtrate chlorohydrine (**3**), were isolated from a dichloromethane fraction that offered 90% protection against crisis-like tonic-clonic seizures in an *in vivo* pharmacological maximal electroshock seizure (MES) test in mice (35 mg/kg, p.o.). According to an *in vitro* GABA-A/BDZ binding site test, the mechanism of action for these compounds does not involve binding to the GABA-A receptor. These compounds are reported in this species for the first time. The valepotriate hydrines isolated from *V. pavonii* could be active metabolites of this species with anticonvulsant properties, however further *in vivo* an in vitro studies are required. Their molecular mechanisms of action are unrelated to the benzodiazepine binding site of the GABA-A receptor.

INTRODUCTION

Plants with medicinal properties are an important source for the discovery of new active metabolites with the potential to treat central nervous system diseases in developed and developing countries [1-3]. In recent reports, extracts and fractions of valepotriates (a family of iridoid esters) obtained from different species of the genus *Valeriana* have shown *in vivo* pharmacological effects in anxiety, sedation, depression and convulsion tests [4-8], furthermore *in vitro* neuroprotective effects attributed to iridoids isolated from a Valeriana species have been recently reported [9].

On the other hand it has been shown that the anxiolytic and sedative effects of active metabolites from *V. officinalis* and *V. wallichii,* including iridoids (valepotriates, especially the epoxy-iridoid type), flavonoids and sesquiterpenoids as valerenic acid, are usually mediated through a GABAergic pathway [10-14]. The benzodiazepine site of the GABA-A receptor is a pharmacological target for active molecules used to treat anxiety, sleep disorders and epilepsy [15,16].

The National Survey of Mental Health, developed on behalf of the World Health Organization (WHO) in 2003, reported that two out of every five people in Colombia exhibited symptoms of at least one mental disease at some point during their lifetime, with anxiety disorders being the most prevalent (19.3%) [17]. The National Epidemiological Study of Neurological Diseases (EPINEURO) reported the prevalence of epilepsy to be 10.3 per 1000 people, standing out as one of the most prevalent neurological diseases in our country and others studied [18]. In

KEY WORDS: Anticonvulsant, GABA-A receptor, Tonic-clonic epilepsy, *Valeriana,* Valepotriate hydrines.

* Author to whom correspondence should be addressed. *E-mail:* segiraldoq@unal.edu.co

ISSN 0326-2383

a more recent study [19], the general prevalence of epilepsy was found to be 11.3 per 1,000 people. Little variation among regions was observed, except that prevalence in the eastern region was 23 per 1,000 people. According to the Report on Epilepsy in Latin America, epilepsy is the most common chronic neurological disorder in the world. It is estimated that 50 million people suffer from this disorder, of which approximately 5 million live in Latin America and the Caribbean. It has also been reported that the prevalence of epilepsy in Latin America is greater than that of developed countries [20].

Considering this situation and the associated neurological diseases observed in Colombia, it is important to gain knowledge about plants that exert an influence on the central nervous system. *Valeriana pavonii* Poepp. & Endl. is a native species which has been used in traditional medicine to treat insomnia and anxiety in Colombia, but its bioactive secondary metabolites have not yet been reported. This species grows naturally in various regions of Colombia and is used in folk medicine as a tranquilizer [21]. Previous phytochemical studies on this plant revealed that iridoids and alkaloids metabolites may be responsible for its pharmacological activity, particularly the anticonvulsant, anxiolytic and antidepressant activities observed in experimental *in vivo* mouse models [22,23].

More than 200 Valeriana species have been reported worldwide, being *V. officinalis* (valerian) the most known and studied; however, the molecular mechanism of action of any of the valerian active metabolites *in vivo* is not clear yet. Within the active metabolites found in different species of the genus *Valeriana* are sesquiterpenes (valerenic acid and derivatives), iridoids (valepotriates), flavonoids, alkaloids and lignans [11,12,24-30]. The present study describes the isolation and identification of three valepotriate hydrines that are reported for the first time in *Valeriana pavonii* (Valerianaceae), which were obtained from a dichloromethane fraction that showed anticonvulsant activity *in vivo*. Anticonvulsant effects have been scarcely studied in species of genus *Valeriana*.

MATERIALS AND METHODS

Plant material

In October 2006 stems of *V. pavonii* (25 kg) were bought from a popular market in Bogotá (plaza de mercado de Paloquemao) that sells medicinal plants. The botanical identification was made at the Colombian National Herbarium of the Universidad Nacional de Colombia (Bogotá, Colombia), and two voucher specimens (Col 495179 and Col 495756) were deposited. Fresh material was fragmented and dried in a forced-air oven at 40 °C for 48 h and then ground for extraction in a disc mill.

Extraction

Dry and crushed stems of *V. pavonii* (400 g) were submitted to a solid-liquid extraction with EtOH (ethanol) at room temperature three times. Each extraction was conducted for 24 h, and the solvent was removed under reduced pressure to obtain the crude ethanolic extract (30.3 g). The ethanolic extract from *V. pavonii* (EEVP: 26.0 g) was initially partitioned into a CH_2Cl_2-H_2O (1:1, v/v) solution. The organic phase was concentrated (11.8 g) and then dissolved in a MeOH-H_2O solution (9:1, v/v, 75 mL), which was then extracted with n-hexane (3 × 25 mL). The aqueous-methanolic phase (1:1, v/v) was submitted to successive extractions with CH_2Cl_2 (3 × 100 mL) to obtain a dichloromethane fraction from *V. pavonii* (DFVP: 1.8 g).

Isolation

The dichloromethane fraction from *V. pavonii* (DFVP: 11.6 g) was subjected to column chromatography (CC) using silica gel 60 (0.063-0.200 mesh, Merck®) and a gradient of increasing polar eluents composed of n-hexane:$CHCl_3$:MeOH (0-100%), yielding thirty major fractions combined according to thin-layer chromatography (TLC) detection, which were grouped in eleven fractions (A-K) based in TLC analysis. Fraction C (4.7 g., 57.6% yield with respect to DFVP) showed a large amount of iridoids by TLC (eluent: $CHCl_3$:MeOH, 9.8:0.2) revealed as blue spots by the specific staining reagent HCl:AcOH (8:2) and was submitted to further purification. The remaining fractions showed complex mixtures of intermediate to high polarity compounds by TLC, and were not included in this work. Fraction C (2.5 g) was submitted to CC on Sephadex LH-20 (20-100 mm, Sigma-Aldrich®), using CHCl3:MeOH (1:1) as the eluent to afford six fractions (C1-C6). Fraction C4 (200.0 mg) was purified by flash CC using silica gel (UV 254 nm/TLC, Macherey Nagel®) and a gradient of increasing polar eluents composed of n-hexane:Et_2O:AcO:MeOH (0-100%), yielding nine fractions (C4a-C4i). Fraction C4-f (33.8 mg) was purified by preparative TLC (silica gel 60/UV 254 nm, 0.25 mm, Macherey Nagel®), eluting first with an n-hexane:AcO (1:1) solution

and then with an n-hexane-Et_2O (1:1) solution to give **1** (10.6 mg).

Fraction C5 (280.6 mg) was purified by flash CC using silica gel (UV 254 nm/TLC, Macherey Nagel®) and a gradient of increasing polar eluents composed of n-hexane:Et2O:AcO:MeOH (0-100%) to obtain twelve fractions (C5a-C5l). Fractions C5b (34.3 mg), C5c (86.5 mg) and C5d (23.5 mg) were initially purified by preparative TLC (SIL-G-100/UV 254 nm, 1 mm, Macherey Nagel®) eluting with an n-hexane:Et_2O (1:1) solution and then were purified by preparative TLC (silicagel 60/UV 254 nm, 0.25 mm, Macherey Nagel®) eluting with a toluene-EtOAc (8:2) solution to give **2** (8.5 mg) and **3** (4.9 mg).

Spectroscopic studies of the isolated compounds were performed using IR analysis (FTIR Bruker® IFS 55). All NMR spectra were run on Bruker Avance® instruments operating at 400 or 500 MHz. Chemical shifts are reported on the d in CDCl3, and scale spaced in parts per million downfield from TMS. Carbon multiplicities were determined by DEPT-135 and DEPT-90 experiments. ^{1}H-^{1}H correlations were observed in COSY and ROESY experiments.

One-bond ^{13}C-1H correlations were observed in an HMQC experiment, and long-range ^{13}C-^{1}H correlations were observed in HMBC experiments. EIMS and HREIMS were obtained on a Micromass Autospec Spectrometer.

In vivo anticonvulsant activity

Maximum electroshock seizure (MES) model of epilepsy was performed according to conditions previously described [31]. In the maximal electroshock test, convulsive seizures are induced by applying an electric current to the brain that is strong enough to initiate a seizure event that spreads throughout the CNS. The induction of clonic and tonic convulsive seizures is indicative of partial or generalized epilepsy in the animal model. Albino (ICR) male mice, 9-11 weeks old and weighing 28-38 g each, were used in the present study. Experimental animals were obtained from the animal house at the Department of Pharmacy, Universidad Nacional de Colombia. The animals were housed in groups at a controlled temperature (22 ± 1 °C) and exposed to a 12 h light/dark cycle, with light between 7 a.m. and 7 p.m. The mice were allowed to consume food and water *ad libitum*. They were divided into groups of 10 and randomly assigned to treatment and control groups. The animals were subjected to a fasting period of no more than 6 h during the test day so that the convulsive threshold would not be affected; the treatments were administered orally (volume: 0.01 mL/g body weight in all cases). EEVP was administered at a dose of 500 mg/kg, p.o., as in previous studies [22,23]. DFVP was administered at a dose proportional to its performance with respect to the EEVP (7.0% = 35 mg/kg, p.o.). Sodium phenytoin (DFH: positive control, Sigma®) was administered at a dose of 20 mg/kg, and the vehicle (negative control: 10% propylene glycol, 10% glycerol, 1% polysorbate-80 and distilled water in sufficient quantity to reach 100%) was administered to the control group. Generalized seizures were induced by electroshock one hour later through corneal electrodes (Coulbourn Instruments®, E 13-51, stimulator) that delivered a 50 mA alternating current of constant frequency (60 Hz) for 0.02 sec to elicit tonic hind-limb extension in the animals. A protective effect was assumed when the drug prevented the tonic extension of the hind limb to an angle greater than 45° [31]. The ratios and percentages of animals protected in each group were determined.

All procedures were conducted following the care principles for the management of laboratory animals (Resolution 8430/1993, Ministerio de la Protección Social in Colombia). The Ethics Committee of the Faculty of Science at the National University of Colombia endorsed this study (Acta 03/2007).

In vitro GABAA/BDZ

The binding of ^{3}H-flunitrazepam (^{3}H-FNZ) to BDZ-bs (81.8 Ci/mmol; New England Nuclear, NEN) in washed crude synaptosomal membranes from rat cerebral cortices was conducted as described by Marder *et al.* [12]. For each of the inhibition experiments, triplicate membrane samples containing 0.2-0.4 mg of protein were suspended in a final volume of 1 mL of 25 mM Tris-HCl buffer, pH 7.3, in the presence of a solution of the sample being tested (compound **1**, **2** or **3**: 300 µM). The samples were incubated at 4 °C for 60 min with 0.4 nM ^{3}H-FNZ. The displacement of ^{3}H-FNZ binding to BDZ-bs by the test compounds was determined with a liquid scintillation counter according to procedures described previously [12].

Statistical analysis

The MES results were expressed as a percentage of the observed protection (index pro-

tection). The Chi² ($p \leq 0.05$) test was applied to MES tests according to their dichotomous responses. Statistical analyses were performed using the statistical software package SPSS (version 15).

RESULTS

Isolation and identification of valepotriate hydrines

Compounds **1** (10.6 mg), **2** (8.5 mg) and **3** (4.9 mg) were obtained as clear yellow viscous liquids from dichloromethane fractions of the *V. pavonii* ethanolic extract (Fig. 1). The IR spectrum of the isolated compounds showed absorption bands due to hydroxy (3484-3472 cm^{-1}), ester carbonyl groups (1738-1739 cm^{-1}) and characteristic bands of double bond in valepotriates diene-type (1611 and 1644 cm^{-1}). The structures of the compounds were elucidated by analysis of 1D and 2D NMR and comparison of their spectral data with those reported in the literature [32]. The molecular formula $C_{22}H_{31}ClO_8$ of **3** was determined by analysis of ^{13}C NMR and HREIMS spectra. The structures of these known compounds were identified as valtrate acetoxyhydrine (**1**), valtrate isovaleroyloxyhydrine (**2**) and valtrate chlorohydrine (**3**).

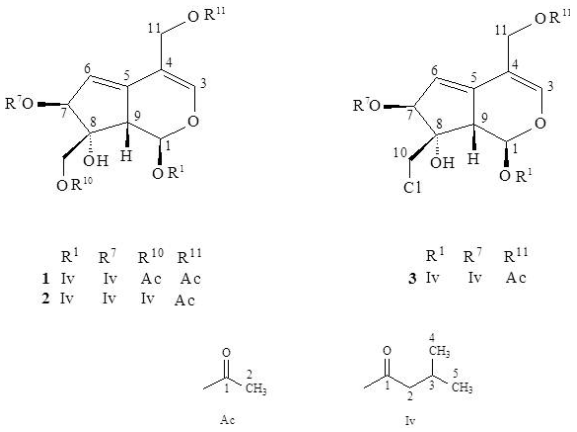

Figure 1. Valtrate acetoxyhydrine (**1**), valtrate isovaleroyloxyhydrine (**2**) and valtrate chlorohydrine (**3**) isolated from *Valeriana pavonii* Poepp. & Endl. Ac: acetyl, Iv: isovaleryl.

In vivo anticonvulsant activity

In the biological test, DFVP showed significant effects on the MES, affording a 90% index of protection in mice at a dose of 35 mg/kg, p.o., whereas EEVP offered only 50% protection, which is consistent with the effects of this extract reported in previous studies (Fig. 2) [22,23]. Phytochemical studies on *V. pavonii* detected an increased amount of blue spots that are indicative of iridoids in DFVP compared to EEVP as measured by TLC.

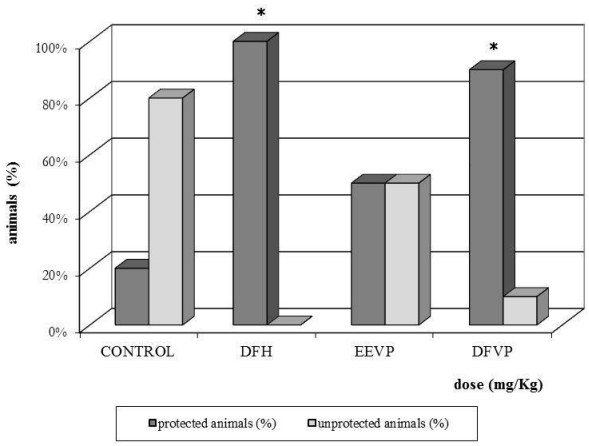

Figure 2. Protection percentages induced in mice by ethanolic extract of *V. pavonii* and dichloromethane fraction in the maximal electroshock seizure test (MES). CONTROL: vehicle; DFH: sodium phenytoin, 20 mg/kg, p.o.; EEVP: ethanol extract, 500 mg/kg, p.o.; DFVP: dichloromethane fraction, 35 mg/kg, v.o.; n: 10, *$p \leq 0.05$, Chi².

In vitro GABA-A/BDZ-binding test

At concentrations of 300 µM, compounds **1**, **2** and **3**, did not fully displace the 3H-FNZ that was bound to the BDZ-bs present in the synaptosomal membranes of rat cerebral cortices. Valtrate acetoxyhydrine (**1**), valtrate isovaleroyloxyhydrine (**2**) and valtrate chlorohydrine (**3**) exhibited 11, 14, and 34% inhibition of ^3H-FNZ binding to BDZ-bs, respectively.

DISCUSSION

The first valepotriates (valeriana-epoxy-triesters) were isolated from *V. wallichii* (valtrate, 1-acevaltrate and didrovaltrate) and are believed to be the bioactive constituents of most *Valeriana* species [24]. High amounts of this compounds are present in *V. edulis* (syn.: *V. Mexican* DC.) (8.0 -12.0%) and *V. wallichii* (syn.: *V. jatamansi* Jones) [33]. Based on their chemical structure, valepotriates can be divided into four main groups: diene-type, monoene-type, valtrate-hydrine-type and desoxy-monoene-type. Valtrate acetoxyhydrine (**1**), valtrate isovaleroyloxyhydrine (**2**) and valtrate chlorohydrine (**3**) are diene-type valepotriate hydrines that were initially isolated by Finner *et al.* from *V. edulis* and *V. wallichii* [32]. These compounds are reported for the first time in *V. pavonii*. Various types of iridoids, including valeriotriates (iridoid

triesters), valeriotetrates, acylated iridoids (jatamanvaltrates), valtrate derivatives and chlorinated valepotriates, have been isolated from the roots and rhizomes of *V. jatamansi* Jones [34-38]. This plant is native to mainland China and India, where it is known as a multipurpose medicinal plant and acts as an important substitute for the European *V. officinalis*. Recent reports attribute the anxiolytic activity of *V. officinalis* and *V. glechomifolia* to the valepotriates present in the fractions tested using the *in vivo* elevated plus maze model [6,7], while the valepotriates isolated from *V. jatamansi* have shown not only sedative activity but also cytotoxic, antitumor and antifungal activities [37,38].

V. pavonii is a native species, used in traditional medicine in different regions of Colombia, in order to treat cases of insomnia and anxiety [21]. Notably, it is often used instead of *V. officinalis,* the best known and studied species in this genus. However, until recently, the active components responsible for its pharmacological effects *in vivo* were not known. Recently, the isolation and identification of isovaleramide from the alkaloidal fraction of *V. pavonii* was reported for the first time in this species. At 100 mg/kg, p.o, this compound exhibited a 90% index of protection against the maximal electroshock seizure test in mice [39].

Valepotriate hydrines isolated from *V. pavonii,* which are present at a relatively high concentration in the DFVP, are active metabolites that contribute to the anticonvulsant effects observed in this study, although synergistic interactions between these compounds or between valepotriate hydrines and alkaloid-type compounds also are a possibility. According to phytochemical studies using TLC, alkaloid-type compounds in *V. pavonii* are also present in the ethanolic extracts at an even higher concentration than in *V. officinalis*.

Although various species of the genus *Valeriana* are being studied because of their pharmacological effects on the central nervous system, their potential for anticonvulsant activity has not been studied a lot *in vivo*. Reports of *in vitro* studies on possible metabolites with this activity are also scarce.

In previous reports on the anticonvulsant activity of the genus *Valeriana,* the activities of ethanolic and other alcoholic extracts from *V. officinalis* and *V. edulis* against chemically induced convulsions have been demonstrated in epilepsy models in mice by employing PTZ (pentylenetetrazol) and picrotoxin as chemical convulsants [5,40]. In a recent report the anticonvulsant *in vivo* effects of aqueous and petroleum ether (PE) extracts from *V. officinalis* were evaluated on the basis of on an experimental model of temporal lobe epilepsy (TLE) in rats. In that study, the results showed a significant anticonvulsant effect for aqueous but not PE extracts of valerian, and it was concluded that part of the anticonvulsant effect of valerian is probably mediated through activation of the adenosine system [41]. In another report that studied the pharmacological effects of alcohol extracts from *V. prionophylla,* protective effects against PTZ-induced convulsions were not observed [42].

In terms of *in vitro* tests, it has been shown that the anxiolytic and sedative effects of active metabolites of *V. officinalis* and *V. wallichii,* including valepotriates (especially the epoxy-iridoid type) and sesquiterpenoids as valerenic acid and flavonoids, are usually mediated through a GABAergic pathway [10-12,43], and other studies have reported mechanisms of action that are related to other receptors, such as serotonin, melatonin, adenosine or glutamate [44-48]. Nevertheless, there are no reports on molecular mechanism of action of valepotriate hydrines isolated from species of the genus *Valeriana*.

The results obtained in this work in the *in vitro* assay confirm that the valepotriate hydrines isolated from *V. pavonii* are not fixed to the benzodiazepine site of GABA-A. It is worth noting that isovaleramide an active compound from *V. pavonii* with anticonvulsant effects, exhibited a 42% of inhibition of the binding of 3H-FNZ to its sites in this same *in vitro* binding test [39].

These results contribute to the pharmacological study of the dichloromethane fraction of *V. pavonii,* and the anticonvulsant effects may be due to valepotriate hydrines acting through mechanisms that are not associated with binding to this receptor. In addition, although valepotriate hydrines isolated from C fraction of *V. pavonii* could have anticonvulsant activity, another active compounds may be present in the fractions left behind and should be analyzed in future studies.

The results achieved in this study contribute to the phytochemical and pharmacological characterization of *V. pavonii* using models of tonic-clonic seizures, in which valepotriates especially valepotriate hydrines, could have anticonvulsant effects and their molecular mechanisms of action are unrelated to the benzodiazepine binding site of the GABA-A receptor. Nevertheless, phytochemical studies are necessary to isolate

alkaloid-type metabolites, which could have synergistic effects with the valepotriate hydrines present in the fractions from *V. pavonii*.

CONCLUSIONS

The valepotriate hydrines isolated from *Valeriana pavonii* Poepp. & Endl. could be active metabolites of this species, with anticonvulsant properties. Their molecular mechanisms of action would be unrelated to the benzodiazepine binding site of the GABA-A receptor. Further *in vivo* and *in vitro* studies are required to identify other receptors or signaling pathways involved in this mechanism of action. These results support the traditional use of *V. pavonii* and validate the interest in this plant as a therapeutic source.

Acknowledgments. The authors wish to thank Colciencias and the Universidad Nacional de Colombia-Bogotá (VRI/DIB, Projects: 8485, 9334, 8035, 5698) for financial support. Thanks to Juliane Hartnack for her English style corrections. Thanks to Animalarium of Pharmacy Department, School of Sciences, UNCSB.

REFERENCES

1. Kumar, V. (2006) Phytother. Res. 20: 1023-35.
2. Gurib-Fakim, A. (2006) *Mol. Aspects. Med.* **27**: 1-93.
3. Gomes N.G., M.G. Campos, J.M. Órfão & C.A. Ribeiro (2009) *Prog. Neuropsychopharmacol. Bio. Psychiatry*. **33**: 1372-89.
4. De Feo V. & C. Faro (2003) *Phytother. Res.* **17**: 661-4.
5. Oliva I., M.E. González-Trujano, J. Arrieta, R. Enciso-Rodríguez & A. Navarrete (2004) *Phytother. Res.* **18**: 290-6.
6. Hadjikhani, R. (2009) *World Acad. Sci. Eng. Technol.* **55**: 532-6.
7. Maurmann N., G.K. Reolon, S.B. Rech, A.G. Fett-Neto & R. Roesler (2009) *Evid. Based. Complement. Alternat. Med.* **2011**: 1-7.
8. Müller L.G., L.A. Salles, A.C. Stein, A.H. Betti, S. Sakamoto, E. Cassel *et al.* (2012) *Prog. Neuropsychopharmacol. Bio. Psychiatry*. **36**: 101-9.
9. Xu J., P. Zhao, Y. Guo, C. Xie, D.Q. Jin, Y. Ma *et al.* (2011) *Fitoterapia* **82**: 1133-6.
10. Mennini T., T. Bernasconi, E. Bombardelli & P. Morazzoni (1993) *Fitoterapia* **64**: 291-300.
11. Wasowski C., M. Marder, H. Viola, J.H Medina & A.C. Paladini (2002) *Planta Med.* **68**: 932-6
12. Marder M., H. Viola, C. Wasowski, S. Fernádez, J.H. Medina & A.C. Paladini (2003) *Pharmacol. Biochem. Behav.* **75**: 537-45.
13. Khom S., I. Baburin, E. Timin, A. Hohaus, G. Trauner, B. Kopp, *et al.* (2007) *Neuropharmacology* **53**: 178-87.
14. Benke D., S. Barberis, S. Kopp, K. Altmann, M. Schubiger, K. Vogt, *et al.* (2009) *Neuropharmacology* **56**: 174-81.
15. Rupprecht R., D. Eser, P. Zwanzger & H.J. Möller (2006) *World J. Biol. Psychia.* **7**: 231-7.
16. Möhler, H.J., (2006) *Cell Tissue Res.* **326**: 505-16.
17. Posada J.A., S.A. Aguilar, C.G. Magaña & L.C. Gómez (2004) *Rev. Col. Psiquiatr.* **33**: 241-62
18. Pradilla G., B. Vesga, F. León-Sarmiento & Grupo Geneco (2003) *Rev. Panam. Salud Publica* **14**: 104-11.
19. Vélez A. & J. Eslava-Cobos (2006) *Epilepsia* **47**: 193-201.
20. Organización Panamericana de la Salud/Organización Mundial de la Salud (2008) "Informe sobre la Epilepsia en Latinoamérica". Liga Internacional Contra la Epilepsia, Buró Internacional para la Epilepsia. Available at: <http://www.ibeepilepsy.org/downloads/Latin%20American%20Regional%20Report%20Spanish.pdf>.
21. García-Barriga, H. (1992) "Flora Medicinal de Colombia", in "Botánica Médica t. 3 (Mundo Editores, ed.), Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, pp. 254-7, 261-2.
22. Arévalo D., C.A. Martínez, J. Rincón & M.F. Guerrero (2006) *Rev. Col. Cienc. Quim. Farm.* **35**: 168-76.
23. Celis C.T., J. Rincón & M.F. Guerrero (2007) *Rev. Col. Cienc. Quim. Farm.* **36**: 11-22.
24. Houghton, P.J. (1999) *J. Pharm. Pharmacol.* **51**: 505-12.
25. Fernández S., C. Wasowski, A.C. Paladini & M. Marder (2004) *Pharmacol. Biochem. Behav.* **77**: 399-404.
26. Chen Y.G., L.L. Yu, R. Huang, Y.P. Lv & S.H. Gui (2005) *Arch. Farm. Res.* **10**: 1161-3.
27. Guo Y., J. Xu, Y. Li, R. Watanabe, Y. Oshima, T. Yamakuni, *et al.* (2006) *Chem. Farm. Bull.* **54**: 123-5.
28. Xu Y., S.P. McLaughlin & A.A. Lesli (2007) *J. Nat. Prod.* **70**: 2045-8.
29. Do K.H., Y.W. Choi, E.K. Kim, S.J. Jun, M.S. Kim, S.Y. Lee, *et al.* (2009) *Phytomedicine* **16**: 530-7.
30. Zhao Y.X., P.Ch. Wang, X.H. Ran, Q.Y. Ma, N. Li, Y.Q. Liu, *et al.* (2011) *J. Chin. Chem. Soc.* **58**: 1-4.
31. Swinyard E., J. Woodhead, H. White & M. Franklin (1989) "Experimental detection, quantification, and evaluation of anticonvulsants", in "Antiepilectic Drugs" (R. Levy, ed.), Raven Press, New York, pp. 111-26.
32. Finner E., S. David & P.W. Thies (1984) *Planta Med.* **50**: 4-6.

33. Bos R., H.J. Woerdenbagb & N. Prasa (2002) *J. Chromatogr. A* **967**: 131-46.
34. Tang Y., X. Liu & B. Yu (2002) *J. Nat. Prod.* **65**: 1949-52.
35. Yu L., R. Huag, Ch. Han, Y. Lv, Y. Zhao & Y. Chen (2005) *Helv. Chim. Acta* **88**: 1059-62.
36. Wang R., D. Xiao, Y.H. Bian, X.Y. Zhang, B.J. Li, L.S. Ding *et al.* (2008) *J. Nat. Prod.* **71**: 1254-7.
37. Lin S., Y.H. Shen, H.L. Li, X.W. Yang, T. Chen, L.H. Lu *et al.* (2009) *J. Nat. Prod.* **72**: 650-5.
38. Lin S., Z.X. Zhang, T. Chen, J. Ye, W.X. Dai, L. Shan, *et al.* (2013) *Phytochemistry* **85**: 185-93
39. Giraldo S.E., J. Rincón, P. Puebla, M. Marder, C. Wasowski, N. Vergel, *et al.* (2010) *Biomedica* **30**: 245-50.
40. Hiller K.O. & G. Zetler (1996) *Phytother. Res.* **10**: 145-51.
41. Rezvani M.E., A. Roohbakhsh, M. Allahtavakoli & A. Shamsizadeh (2010) *J. Ethnopharmacol.* **127**: 313-8.
42. Holzmann I., V.C. Filho, T.C. Mora, A. Cáceres, J.V. Martínez, S.M. Cruz *et al.* (2011) *Evid. Based Complement. Alternat. Med.* **2011**: 1-9.
43. Felgentreff F., A. Becker, B. Meier & A. Brattströmc (2012) *Phytomedicine* **19**: 1216-22.
44. Abourashed E.A., U. Koetter & U. Brattström (2004) *Phytomedicine* **11**: 633-8.
45. Dietz B., G. Mahady, G. Pauli & N. Farnsworth (2005) *Mol. Brain Res.* **138**: 191-7.
46. Lacher S.K., R. Mayer, K. Sichardt K, K. Nieber & C.E. Müller (2007) *Biochem. Pharmacol.* **73**: 248-58.
47. Sichardt K., Z. Vissiennon, U. Koetter, A. Brattström & K. Nieber (2007) *Phytother. Res.* **21**: 932-7.
48. Del Valle L.M., Y.M. Ayala, C.M. Ortiz, B.A. Torres, S.A. Mukhaimer & J.G. Ortíz (2011) *Evid. Based. Complement. Alternat. Med.* **2011**: 1-7.