Isolation of bergenin from the root bark of Securinega virosa and evaluation of its potential sleep promoting effect

Mohammed Garba Magaji1*, Aliyu Muhammad Musa2, Musa Ismail Abdullahi3, Jamilu Ya’u1, Isa Marte Hussaini4,5

1Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria
2Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria
3Department of Pharmaceutical and Medicinal Chemistry, Usman Danfodiyo University, Sokoto-Nigeria
4Department of Pharmacology, University of Maiduguri, Maiduguri-Nigeria
5Department of Pathology, University of Virginia, USA

Abstract

Objectives: Securinega virosa Roxb (Ex Willd) Baill (Euphorbiaceae) root bark has been reportedly used in African traditional medicine in the management of mental illnesses. Previously, the sleep-inducing potential of the crude methanol root bark of Securinega virosa extract and its butanol fraction have been reported. The study aimed to isolate and characterize the bioactive constituent that may be responsible for the sleep inducing property of the root of the plant.

Materials and Methods: The phytochemical investigation of the S. virosa root bark was carried out leading to the isolation of a compound from the butanol-soluble fraction of the methanol extract. The structure of the compound was elucidated on the basis of its spectral data, including IR, 1D and 2D NMR, mass spectrometry as well as X-ray diffraction analysis. The compound was investigated for sleep-inducing potential using diazepam-induced sleeping time test and beam walking assay in mice.

Results: This is the first report on the isolation of bergenin from the root of the plant. It significantly decreased the mean onset of sleep \( [F (2, 15) =7.167; p< 0.01] \) at the dose of 10 mg/kg, without significantly affecting the total sleep duration \( [F (2, 15) = 0.090, p=0.914] \). Conversely, it did not significantly affect the number of foot slips at the doses of 5 and 10 mg/kg tested.

Conclusion: Bergenin isolated from the root bark of S. virosa possesses sleep-inducing property and could be partly responsible for the sedative potential of the root of S. virosa.

Keywords:
Securinega virosa
Bergenin
Sleep
Isocoumarin

Introduction

According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioral disorders, yet only a small minority of them receive even the most basic treatment. This amounts to 12.3% of the global burden of disease, and is speculated
Insomnia is a common complaint of inadequate sleep affecting 15-40% of the world population, which complicates several disorders and of which less than 15% receives appropriate treatment (Jiang et al., 2007). The statistics are thought to be higher due to existence of compelling evidences of under-diagnosis, under-recognition and under-treatment of the condition (Najib, 2006). Insomnia is both a risk factor and precursor of behavioral disorder such as depression and, therefore, its effective treatment is essential in the prevention of the major depressive illnesses (Forde and Kamerow, 1989; Eaton et al., 1995). The existing agents used in the management of insomnia which include the benzodiazepines and non-benzodiazepine drugs are associated with untoward effects such as day time fatigue, cognitive impairment and physical dependence. This has necessitated the affected patients to patronize herbal remedies with claims of lower incidence of adverse effects.

The reliance of patients on herbal remedies for the management of their neuropsychiatric disorders is one of the several reasons encouraging ethnopharmacological researches towards the development of potential therapeutic agents with better safety and efficacy profiles for the management of neurological conditions (Zhang, 2004). The potentials of a number of these medicinal plants in the management of neuropsychiatric disorders have been investigated using batteries of behavioral models. Some of these medicinal plants are promising sources of therapeutic agents in the management of CNS disorders.

Securinega virosa is one of the African medicinal plants described as a true “cure all”, of which all parts are used as remedies, particularly the root. The common vernacular names in Nigeria include “tsuwaawun karee, gussu, gwiiwar karee” (Hausa), “iranje” (Yoruba), “njisinta” (Ibo), “shim shim” (Kanuri), “kartfi-kartfi” (Shuwa arabs) and “camal, cambe, came” (Fulani) (Neuwinger, 1996). The decoction of the root with other plant is used in northern Nigeria for the treatment of mental illness. The crude methanolic root bark extract of the plant has been reported to possess sedative activity in laboratory animals (Magaji et al., 2008). Similarly, the neuropharmacological activities of various fractions of the methanol root bark extract of the plant have been reported (Magaji et al., 2013; Magaji et al., 2014a, b). A number of alkaloids have been isolated from the root bark of S. virosa. These include hordenine, norsecurinine (Iketubosin and Mathieson, 1963), dihydronorsecurinine, viroallosecurinine (Saito et al., 1964) and 14, 15-epoxynorsecurinine (Dehmlow et al., 1999). Friedelin, epifriedelanol, stigmasterol and betulinic acid were isolated from the leaves and twig of the plant (Monkodkaew et al., 2009). The aim of the present study, therefore, is to isolate the potential sleep-inducing compound from the methanolic extract of root bark of S. virosa. In this study, we report, for the first time, the isolation of bergenin from the root of S. virosa and its sleep-inducing potential.
weight was obtained. It was then crushed into coarse powder with a pestle and mortar.

**Animals**

Male Swiss Albino mice weighing 18-22 g (7-8 weeks old), were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were maintained in a well-ventilated room in the animal house. They were fed on standard laboratory animal feed and water ad libitum. All experiments performed on laboratory animals in this study were in accordance with Ahmadu Bello University Research policy as well as ethic and regulations governing the care and use of experimental animals as contained in “Principles of laboratory animal care” published by the National Institute of Health (NIH Publication No. 85-23, revised, 1996). The experiments were conducted in quiet laboratory between hours of 9:00 and 16:00.

**Extraction and fractionation**

The powered root bark (1000 g) was extracted with 2 l methanol (absolute) for 72 hours using soxhlet extraction apparatus. The solvent was evaporated on a water bath at 40°C to give a dark brownish residue (Average yield: 9.82 %w/w) which was subsequently referred to as crude methanolic root bark extract. Then, 50 g of the crude methanolic root bark extract was solubilized in distilled water and filtered. The filtrate was successively partitioned with petroleum ether, chloroform, ethyl acetate and n-butanol to yield petroleum ether, chloroform, ethyl acetate and n-butanol-soluble fractions, respectively.

**Chromatographic techniques**

Three grams (3 g) of butanol fraction was chromatographed on silica gel column eluting with ethylacetate (100%), ethyl acetate/ methanol mixtures (95:5, 90:10, 80:20, 70:30 and 50:50) and methanol 100% as solvent systems to give three major fractions when pooled together based on similarity in their thin layer chromatographic (TLC) profile coded Pooled fraction A (PFA), Pooled fraction B (PFB) and Pooled fraction C (PFC). Repeated Sephadex LH-20 gel filtration chromatography using methanol as eluent led to the isolation of a compound coded MAG from the most active fraction, PFA. Thin layer chromatography (TLC) was performed using silica gel 60 F254 (Merck); Column chromatography was performed using Merck silica gel (60–120) mesh and gel filtration chromatography was performed using Sephadex LH-20 (Sigma, USA). Spots on TLC plates were visualized by spraying 10% H2SO4 followed by heating at 100 °C for 5min or with Gibbs reagent.

**Spectral analysis**

NMR-spectra were recorded on a Bruker AVANCE spectrometer (400 MHz) for 1H and (100 MHz) for 13C-NMR using residual solvent peak as internal standard; deuterated methanol was used as solvent and chemical shift values (δ) were reported in parts per million (ppm). The IR spectrum was measured on a Shimadzu FT-IR8 400S Fourier Transform Infrared spectrophotometer. The UV spectrum was recorded by a Hitachi U-3200 spectrophotometer. Melting point was determined using a Yanaco MP-400 micro melting point apparatus. X-ray crystallography was obtained at low temperature from ethyl acetate crystals. The results obtained were compared with those reported in the literature and databases.

**Pharmacological studies**

* Diazepam-induced sleep in mice
  
  The method described by Beretz et al. (1978) and modified by Rakotonirina et al. (2001) was adopted. Initially, the butanol soluble fraction (75, 150 and 300mg), PFA (2.5, 5 and 10 mg/kg), PFB (2.5, 5 and 10
mg/kg) and PFC (2.5, 5 and 10 mg/kg) were subjected to the diazepam induced sleep test. For the isolated compound, mice of either sex were randomly divided into three groups each containing 6 mice. The first group received normal saline (10 ml/kg). The second and third groups received bergenin 5 and 10 mg/kg, respectively. Thirty minutes post-treatment, diazepam at a dose of 25 mg/kg body weight was administered to the mice. The mice were placed individually in cages. The onset and the duration of sleep were determined for each animal. Loss of righting reflex was considered as the criterion for sleep (Rolland et al., 1991) while the interval between the loss and the recovery of straightening was regarded as the duration of sleep (Fujimori, 1965).

**Mouse Beam walking Assay in mice**

The method previously described by Stanley et al. (2005) was adopted with slight modification (Magaji et al., 2008). Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Three trials were performed for each mouse. Trials were designed in a way that the mouse tested would be aware of the presence of a goal box that could be reached. The goal box was a Perspex glass cage (with wood chippings beddings) with a small hole at the bottom. The mice that successfully walked along the ruler were randomly grouped into five groups each containing six mice. The first group received normal saline (10 ml/kg), i.p. The second and third groups received bergenin 5 and 10 mg/kg, respectively. The fourth group received diazepam (2 mg/kg body weight). The beam was made of wood, 8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal support. Thirty (30) minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 seconds allowed on beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded.

**Statistical analysis**

Results of the pharmacological studies were expressed as mean ± standard error of mean. Statistical analysis was performed by One-Way analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, a post-hoc Dunnets t-test was performed for multiple comparisons. Values of P<0.05 were considered significant.

**Results**

MAG was obtained as colourless crystals (21.5mg) and was characterized by comparison with spectral data reported in the literature; m.p 192–195°C; UV (MeOH) λ_max: 265, 320nm; IR (KBr): ν_max 3350, 1680 cm^{-1} representing OH and carbonyl functional groups respectively (Dung et al., 2003). HREIMS measurements yielded an ion [M-H]^{-}(m/z=327) corresponding to C_{14}H_{16}O_{9} and the fragmentation pattern is in agreement with that of bergenin (Dung et al., 2003; Li et al., 2013). The 1 & 2D NMR experiments including 1^H, 13C, 1H-1H, HSQC, HMBC and NOIESY data were in agreement with those reported in the literature (Dung et al., 2003; Nasser et al., 2009; da Silva et al., 2009; Nunomura et al., 2009) for bergenin (Figure 1).

![Figure 1. Bergenin](image.png)
Isolation of bergenin from *Securinega virosa* and evaluation of its sleep promoting effect

The x-ray crystallographic analysis of MAG established the stereochemistry (Figure 2) as previously reported (Caldas et al., 2002).

**Pharmacological Studies**

Fraction PFA significantly decreased the mean onset of sleep \([F (3, 20) = 12.572, P < 0.001]\) without affecting the total duration \([F (3, 20) = 2.213, P = 0.118]\) (Figure 3B). Fraction PFB non-dose-dependently increased the total sleep duration (Figure 3C) while fraction PFC dose-dependently increased the total sleep duration (Figure 3D). Bergenin, similar to the PFA from where it was isolated, significantly decreased the mean onset of diazepam induced sleep \([F (2, 15) = 7.167; P < 0.01]\) without affecting the total sleep duration \([F (2, 15) = 0.090, P=0.914]\) (Figure 4).

![Figure 2. View of bergenin showing the x-ray analysis of crystal monohydrate](image)

![Figure 3. Effect of n-butanol soluble fraction of methanolic extract of root bark of *Securinega virosa* and its various fractions on diazepam-induced sleep in mice; Data presented as mean ± SEM; NBF (n-butanol fraction); PFA (Pooled fraction A) PFB (Pooled fraction B); PFC (Pooled fraction C); *p < 0.05 and **p < 0.001 compared to control; n = 6.](image)
In the beam walking assay, while bergenin did not affect the number of foot slips, diazepam (2 mg/kg) significantly increased the total number of foot slips (Figure 5).

**Discussion**

The present study reports the isolation of bergenin from the root of *S. virosa* and its sleep inducing property, for the first time. Bergenin has been isolated from several plants (See Patel et al., 2012 for a review). It has also been isolated from the leaves of *S. virosa* (Sanogo et al., 2009). Its isolation from the root of the plant in this study suggests that it is also an important phytochemical found in *S. virosa* in addition to the securinine alkaloids. Bergenin isolated from different plants have been found to possess antioxidant (Takahashi et al., 2003), anti-inflammatory (Nazir et al., 2007), antiretroviral (Piacente et al., 1996), anti-arrhythmic (Pu et al., 2002) and hepatoprotective (Lim et al., 2000) properties. However, to the best of our knowledge, there is no data in the literature on the effect of bergenin on the central nervous system.

Diazepam-induced sleep model has been used as a preliminary method to study CNS active compounds (Danjuma et al., 2013). Compounds that decrease the onset of sleep have sleep-inducing property while those that increase the total diazepam sleep duration are known to produce general CNS depression (Perez, 1998; Rakotonirina et al., 2001). Previously, the crude methanolic extract of root bark of *S. virosa* has been reported to affect both indices of sleep (onset and duration) in diazepam-induced sleep paradigm (Magaji et al., 2008). Conversely, the butanol fraction of the crude extract was found to decrease the sleep onset without significantly affecting the total sleep duration (Magaji et al., 2012). In this study, we found out that bergenin behaved similar to the n-butanol fraction from which it was isolated by decreasing the onset of sleep without significantly affecting the total sleep duration, suggesting that it may contribute to the sleep-inducing property of the extract, since other phytochemical such as flavonoids, saponins and alkaloids found in the extract and fraction have been reported to have sleep-modulating properties. The roles of various
endogenous neurotransmitters in the sleep have been well documented. The dopaminergic neurotransmission is involved in the maintenance of behavioral alertness and waking mechanism while a decrease in dopaminergic receptor activity has been found to produce a state of sleepiness (Osuide and Wambebe, 1980; Gillin et al., 2000). Dopamine D2 receptor have been reported to play an essential role in the maintenance of wakefulness but not in homeostatic regulation of non-rapid eye movement sleep (NREM) (Qu et al., 2010). Conversely, the dopamine D1 receptors are involved in the regulation of rapid eye movement (REM) sleep process (Trampus et al., 1991). The benzodiazepines produce their sedative, anxiolytic, muscle relaxant, anticonvulsant as well as cognitive impairment through potentiation of GABAergic neurotransmission. Specifically, the benzodiazepines are known to allosterically modulate the a1 subtype of the GABA_A receptor to produce their sedative effect (McKernan et al., 2000).

The exact mechanism via which bergenin reduces the mean onset of diazepam-induced sleep was not elucidated here. However, the activity of bergenin in reduction of sleep onset may be similar to that of ramelteon, a chronohypnotic that acts on the melatonin MT1 and MT2 receptors in the suprachiasmatic nucleus which promotes sleep onset without the characteristic side effects (cognitive impairment, motor disturbance, dependence, tolerance, hangover, and rebound insomnia) associated with the GABA_A receptor modulating agents (Miyamoto, 2009).

Warfarin, a coumarin analogue was found to increase hexobarbital-induced sleeping time (Apseloff et al., 1991). Conversely, the 4-hydroxy coumarin isolated from the Viola betonicifolia was found to be devoid of sleep potentiating property (Muhammad et al., 2013), suggesting that the CNS activity of coumarins may not be general but seems to be dependent on the chemistry of the compounds. Coumarin and derivatives have been reported to be neuroprotective (Kang et al., 2005; Kang and Kim, 2007; Epifano et al., 2008). Bergenin has also been reported to possess antioxidant and anti-inflammatory potentials (Takahashi et al., 2003; Nazir et al., 2007) and could therefore possess neuroprotective activity.

The Beam walking assay is a more sensitive tool than the rota rod in determination of benzodiazepine-induced motor coordination deficit (Stanley, 2005). Compounds that induce motor coordination deficit are known to increase the number of foot slips when compared to the control. The time to complete the task and the number of falls are not as sensitive as the number of foot slips. The inability of bergenin to increase the number of foot slips suggest that it may not interfere with cerebellar-dependent motor coordination at the doses used in the study (Otte et al., 2009); and that its action may be limited to the limbic area of the brain that control arousal.

In conclusion, bergenin isolated from the root bark of S. virosa possesses sleep-inducing property. Study is currently going on to predict the possible mechanism(s) by which bergenin influences the CNS using different behavioral paradigms of neuropsychiatric disorders, some of which have oxidative imbalance and neuronal excitation pathologies.

Acknowledgement
Authors wish to acknowledge the Department of Pathology, University of Virginia, USA for the MS, the X-ray crystallography unit, School of Physics, Universiti Sains Malaysia for Diffraction Analysis and the Department of Chemistry, University of Pretoria, SA for running the NMR spectra.

Conflict of Interest
The authors report no conflicts of interest.
References

Apseloff G., Hilliard JB, Gerber, N, Mays DC. 1991. Inhibition and induction of drug metabolism by psoralens: alterations in duration of sleep induced by hexobarbital and in clearance of caffeine and hexobarbital in mice. Xenobiotica, 21: 1461-1471.

Beretz A, Haag-Berrurie RM, Anton R. 1978. Choice of pharmacological methods for the study of the activities of the hawthorn. Med Plant Herbal Med, 4: 305-314.

Caldas CS, De Simone CA, Pereira MA, Malta VRS, Carvalho RLP, Da Silva TBC, Sant'ana AEG, Conserva LM. 2002. Bergenin monohydrate, a constituent of Hurmiria balsamifera, at 120 K. Acta Cryst, 58: o609-o611.

Danjuma NM, Abdu-Aguye I, Anuka JA, Hussaini IM, Zezi AU. 2009. Evaluation of anticonvulsant activity of the hydroalcoholic stem bark extract of Randia nilotica Stapf. in mice and chicks. Nig J Pharm Sci, 8: 36-45.

Dehmlow EV, Guntenhoner M, Ree TV. 1999. A novel alkaloid from Fluggea virosa: 14, 15-epoxynorsecurinine. Phytochemistry, 52: 1715-1716.

Dung NT, Ty PE, Taylor WC, Hoang VE. 2004. Bergenin isolated from Ficus glomerata bark. Vietnam J Chem, 42: 250-252.

Eaton WE, Badawi M, Melton B. 1995. Prodromes and precursors: epidemiologic data for primary prevention of disorders with slow onset. Am J Psychiatry, 152: 967-972.

Epifano F, Molinaro G, Genovese S, Ngomba RT, Nicoletti F, Curini M. 2008. Neuroprotective effect of prenyloxycoumarins from edible vegetables. Neurosci lett, 443: 57-60.

Ford DE, Kamerow DB. 1989. Epidemiologic study of sleep disturbances and psychiatric disorders: an opportunity for prevention? JAMA, 262: 1479-1484.

Fujimori H. 1965. Potentiation of barbital hypnosis as an evaluation method of central nervous system depressant. Psychopharmacology, 7: 374-397.

Gillin CJ, Seifritz E, Zolotoski RK. 2000. Basic science of sleep In: Sadock BJ, Sadock VA eds, Kaplan and Sadock's Comprehensive Textbook of Psychiatry (7th edition), pp. 199–209, Philadelphia: Lippincott Williams and Wilkins.

Ikotubosin GO, Mathieson DW. 1963. The isolation of hordenineandnorsecurinine from Securinega virosa. The structure of norsecurinine. J Pharm Pharmacol, 15: 810-815.

Jiang JG, Huang XJ, Chen J, Lin QS. 2007. Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen Ziziphus jujube. Nat Prod Res, 21: 310-320.

Kang SY, Kim YC. 2007. Neuroprotective coumarins from the root of Angelica gigas: Structure-activity relationships. Arch Pharmacal Res, 30: 1368-1373.

Kang SY, Lee, KY, Sung SH, Kim YC. 2005. Four New Neuroprotective Dihydropyranocoumarins from Angelica gigas. J Nat Prod, 68: 56-59.

Li BH, Wu JD, Li XL. 2013. LC–MS/MS determination and pharmacokinetic study of bergenin, the main bioactive component of Bergenia purpurascens after oral administration in rats. J Pharmaceut Anal, 3: 229-234.

Lim HK, Kim HS, Choi HS, Oh S, Choi J. 2000. Hepatoprotective effects of bergenin, a major constituent of Mallotus japonicus, on carbon tetrachloride-intoxicated rats. J Ethnopharmacol, 72: 469-474.

Magaji MG, Anuka JA, Abdu-Aguye I, Yaro AH, Hussaini IM. 2008. Behavioural Effects of the methanolic root bark extract of Securinega virosa in rodents. Afr J Tradit Complement Altern Med, 5: 147-153.

Magaji MG, Mohammed M, Magaji RA, Musa AM, Abdu-Aguye I, Hussaini IM. 2014. Evaluation of the Antipsychotic Potential of Aqueous...
Isolation of bergenin from Securinega virosa and evaluation of its sleep promoting effect

Fraction of Securinega virosa Root Bark extract in mice. Metab Brain Dis, 29: 161-165

Magaji MG, Yakubu Y, Magaji RA, Yaro AH, Hussaini IM. 2014. Psychopharmacological potentials of Methanol leaf extract of Securinega virosa Roxb (Ex Willd) Baill. in mice. Pak J Biol Sci, 17: 855-859.

Magaji MG, Yaro AH, Musa AM, Anuka JA, Abdu-Aguye I, Hussaini IM. 2013. Neuropharmacological studies on ethyl acetate fraction of Securinega virosa root bark extract. Afr J Pharm Pharmacol, 7: 275-279.

Magaji MG, Yaro, AH, Musa, AM, Anuka, JA, Abdu-Aguye, I and Hussaini, IM. 2012. Central Depressant Activity of butanol fraction of Securinega virosa root bark in mice. J Ethnopharmacol, 141: 128-133.

McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABAA receptor α1 subtype. Nature Neurosci, 3: 587-592.

Miyamoto M. 2009. Pharmacology of ramelteon, a selective MT1/MT2 receptor agonist: a novel therapeutic drug for sleep disorders. CNS Neurosci Ther, 15: 32-51.

Monkodkaew S, Loetchutinat C, Nuntaesaen N, Pompimon W. 2009. Identification and Antiproliferative Activity Evaluation of a Series of Triterpenoids Isolated from Flueggea virosa (Roxb. ex Willd). Am J Appl Sci, 6: 1800-1806.

Muhammad N, Saeed M, Khan H, Adhikari A, Khan KM. 2013. Muscle Relaxant and Sedative-Hypnotic Activities of Extract of Viola betonicifolia in Animal Models Supported by Its Isolated Compound, 4-Hydroxy Coumarin. J Enzyme Inhib Med Chem, 28: 997-1001

Najib J. 2006. Eszopiclone, a nonbenzodiazepine sedative-hypnotic agent for the treatment of transient and chronic insomnia. Clin therap, 28: 491-516.

Nasser JA, Yaacob WA, Din LB, Yamin BM, Latip J. 2009. Isolation of atranorin, bergenin and goniotothalamin from Hopea sangal. ARPN J Eng Appl Sci, 4: 92-95.

Nazir N, Koul S, Qurishi MA, Taneja SC, Ahmad SF, Bani S, Qazi GN. 2007. Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis—A flow cytometric study. J Ethnopharmacol, 112: 401-405.

Neuwinger JD. 1996. (translated from the German by Porter, A.). African ethnobotany-poisons and drugs, pp. 495-499, Weinheim, Germany: Chapman and Hall.

Nunomura R, Oliveira VG, Da Silva SL, Nunomura SM. 2009. Characterization of bergenin in Endopleura uchi bark and its anti-inflammatory activity. J Braz Chem Soc, 20: 1060-1064.

Ouside G, Wambebe, C. 1980. Antagonism of pentobarbitone sleep by dopamine, levodopa and apomorphine in chicks. Clin Exp Pharmacol Physiol, 7: 237-248

Otte DM, Bilkei-Gorzó A, Filiou MD, Turck CW, Yilmaz Ö, Holst MI, Schilling K, Abou-Jamra R, Schumacher J, Benzel I, Kunz WS, Beck H, Zimmer A. 2009. Behavioral changes in G72/G30 transgenic mice. Eur Neuropsychopharmacol, 19: 339-348.

Patel DK, Patel K, Kumar R, Gadewar M, Tahilyani V. 2012. Pharmacological and analytical aspects of bergenin: a concise report. Asian Pac J Trop Biomed, 2: 163-167.

Perez GRM, Perez LJA, Garcia D LM, Sossa M H. 1998. Neuropharmacological activity of
Solanum nigrum fruit. J Ethnopharmacol, 62: 43-48.
Piacente S, Pizza C, De Tommasi N, Mahmood N. 1996. Constituents of Ardisia japonica and their in vitro anti-HIV activity. J Nat Prod, 59: 565-569.
Pu HL, Huang X, Zhao JH, Hong A. 2002. Bergenin is the Antiarrhythmic Principle of Fluggea virosa. Planta Med, 68: 372-374.
Rakotonirina SV, Ngo Bum E, Rakotonirina A Bopelet M. 2001. Sedative properties of the decoction of the rhizome of Cyperus articularis. Fitoterapia, 72: 22-29.
Reynolds EH. 2003. Brain and mind: a challenge for WHO. Lancet, 361: 1924–1925.
Rolland A, Fleurentain J, Lan hers M, Younos C, Misslin R, Morier F. 1991. Behavioural effects of American traditional plant. Eschscholzia California: Sedative and anxiolytic properties. Planta Med, 57: 212-216.
Saito S, Tanaka T, Kotera K, Nakai H, Sugimoto N, Horii Z, Ikeda M, Tamura Y. 1964. Structure and stereochemistry of norsecurinine and dihydronorsecurinine. Chem Pharm Bull, 12: 1520-1523.
Sanogo R, Vassallo A, Malafron te N, Imparato S, Russo A, Dal Piaz F. 2009. New phenolic glycosides from Securinega virosa and their antioxidant activity. Nat Prod Commun, 4: 1645-1650
Silva SLD, Oliveira VGD, Yano T, Nunomura RDCS. 2009. Antimicrobial activity of bergenin from Endopleura uchi (Huber) Cuatrec. Acta Amaz, 39: 187-191.
Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS. 2005. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol, 19: 221-227.
Takahashi H, Kosaka M, Watanabe Y, Nakade K, Fukuyama Y. 2003. Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. Bioorg Med Chem, 11: 1781-1788.
Trampus M, Ferri N, Monopoli A, Ongini E. 1991. The dopamine D1 receptor is involved in the regulation of REM sleep in the rat. Eur J Pharmacol, 194: 189-194.
Qu WM, Xu XH, Yan MM, Wang YQ, Urade Y, Huang ZL. 2010. Essential role of dopamine D2 receptor in the maintenance of wakefulness, but not in homeostatic regulation of sleep, in mice. J Neurosci, 30: 4382-4389.
WHO. 2001. The World Health Report. Mental health: new understanding new hope. Geneva, PP.1-15.
Zhang ZJ. 2004. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders, Life Sci, 75: 1659–1699.