SEDATIVE ACTIVITY OF SOME PLANTS USED IN MEXICO TO TREAT INSOMNIA

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ABSTRACT

Insomnia is one of the most common sleep disorders around the world. Species of plants such as *Citrus sinensis, Citrus limon*, *Ternstroemia pringlei, Ternstroemia sylvatica*, *Casimiroa edulis*, *Galphimia glauca* and *Cymbopogon citratus* are traditionally and widely used in Mexico as sleep aids. The aim of this work was to evaluate the sedative effect of different extracts of these plants by using the exploratory cylinder model in mice. Different doses of the hexane, dichloromethane, methanol or hydroalcoholic extracts were intraperitoneally administered 30 min before testing in order to obtain their sedative ED_{50} values. The most active extracts were *Galphimia glauca* methanol extract (ED_{50} = 22.06 ± 5.6 mg/kg) and *Cymbopogon citratus* hexane extract (ED_{50} = 27.01 ± 2.9 mg/kg), followed by methanol extract obtained from the *Citrus sinensis* leaves (ED_{50} = 38.48 ± 8.0 mg/kg) and flowers (ED_{50} = 47.04 ± 12.0 mg/kg); whereas, *Ternstroemia sylvatica* methanol extract (ED_{50} = 61.88 ± 6.42 mg/kg), *Galphimia glauca* hexane extract (ED_{50} = 76.85 ± 20.9 mg/kg) and *Cymbopogon citratus* dichloromethane extract (ED_{50} = 77.11 ± 15.0 mg/kg) show lower sedative activity. Other extracts showed an ED_{50} value over 100 mg/kg or were inactive. Our results give evidence of the popular use of these medicinal plants as sleep aids.

Key words: Sedative effect; Mexican traditional medicine; Sleep aids; Sedative plants.

RESUMEN

El insomnio es el desorden del sueño más común en el mundo. Las especies *Citrus sinensis*, *Citrus limon*, *Ternstroemia pringlei*, *Ternstroemia sylvatica*, *Casimiroa edulis*, *Galphimia glauca* y *Cymbopogon citratus* son utilizadas ampliamente y tradicionalmente en México como inductoras de sueño. El objetivo de este trabajo fue evaluar el efecto sedante de diferentes extractos de las plantas antes mencionadas utilizando el modelo de cilindro de exploración en ratones. Para obtener los valores de DE_{50} sedante se administraron por vía intraperitoneal varias dosis de los

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extractos de hexano, de diclorometano, de metanol o hidroalcóholico, 30 minutos antes de ser evaluados. El extracto más activo fue el metanólico de *Galphimia glauca* (DE_{50} = 22.06 ± 5.6 mg/kg), seguido del extracto hexánico de *Cymbopogon citratus* (DE_{50} = 27.01 ± 2.9 mg/kg), los extractos metanólicos de hojas (DE_{50} = 38.48 ± 8.0 mg/kg) y de flores (DE_{50} = 47.04 ± 12.0 mg/kg) de *Citrus sinensis*, el metanólico de *Ternstroemia sylvatica* (DE_{50} = 61.88 ± 6.42 mg/kg) y el extracto de diclorometano de *Cymbopogon citratus* (DE_{50} = 77.11 ± 15.0 mg/kg). Los otros extractos presentaron un valor de la DE_{50} mayor a 100 mg/Kg. Nuestros resultados dan soporte al uso popular de las especies estudiadas como inductoras de sueño.

Palabras clave: Efecto sedante, Medicina tradicional mexicana, inductor del sueño, plantas sedantes

INTRODUCTION

Insomnia is a frequent problem that affects people of all the ages around the world. It is a prevalent and potentially serious condition that adversely affects the diurnal functioning, health status and life quality of people of all of age (Edinger and Means, 2005). Stress, anxiety and depression could trigger insomnia. As well as cough, chronic pain, apnea, circadian rhythm disorders and neural diseases are conditions also associated with insomnia (Harvey, 2001). Insomnia is treated pharmacologically and non-pharmacologically or with a combination of both (Benca, 2005). Benzodiazepines, zolpidem, zopiclone, zaleplon, have been used for the treatment of insomnia (Gottesmann, 2002). The over the counter (OTC) medicines containing the H_1 antagonist diphenhydramine, are also used for treatment of occasional insomnia (Shigemoto *et al.*, 2004). Relaxation, stimulus control, sleep restriction and sleep hygiene are common behavioral therapies for insomnia (Nau *et al.*, 2005).

Medicinal plants constitute another option to treat insomnia. Around the world have been used several plants like sleep inducers, such as Valerian, Passion flower, Melissa, Hops and Kava-Kava (banned) (Wheatley, 2005). Some of the plants more commonly used in Mexico as tranquilizers and sleep aids are "valeriana" (*Valeriana edulis* ssp *procera*), flowers and leaves of *Citrus sp*, "flor de tila" (*Ternstroemia pringlei* and *Ternstroemia sylvatica*), "zapote blanco" (*Casimiroa edulis*), "té limón" (*Cymbopogon citratus*), "toronjil" (*Agastache mexicana*), "calderona amarilla" (*Galphimia glauca*), "ruda" (*Ruta chalepensis*) and "limón" (*Citrus limon*) (Balderas *et al.*, 2008; Instituto Nacional Indigenista, 1994; Tortoriello and Romero, 1992; Ugalde *et al.*, 2005).

The present work describes the screening study of the sedative activity of different extracts of *Casimiroa edulis*, *Citrus limon*, *Citrus sinensis*, *Cymbopogon citratus*, *Galphimia glauca*, *Ternstroemia pringlei* and *Ternstroemia sylvatica*, using the exploratory cylinder test in mice, with the aim to provide experimental support to its traditional use.

MATERIALS AND METHODS

Plant material

The fruits of *Ternstroemia pringlei* were collected in Zirahuato, Michoacán, Mexico and fruits of *Ternstroemia sylvatica* were collected in Montellano, Puebla, Mexico; *Citrus sinensis* flowers and leaves were obtained from orange producers from El refugio, Ciudad Fernández, San Luis Potosí, Mexico. The leaves of *Cymbopogon citratus* and seeds of *Casimiroa edulis* were acquired from a local market (Mercado So-

nora, Mexico City). The homogeneity and authenticity of the plant materials were certified by one of the authors (A. Aguilar), botanist from the Herbarium of the Instituto Mexicano del Seguro Social. Samples of these materials were deposited in the Herbarium of Instituto Mexicano del Seguro Social. *Galphimia glauca* was collected in Tehuetlán, Hidalgo, Mexico. A sample of this material was deposited at Herbarium of the Universidad Autónoma Chapingo. Voucher numbers for each plant sample are indicated in Table 1. The dry peel of *Citrus limon* was donated by Deshidratadora Tecomense S.A de C.V., Colima, Mexico. A commercial sample of powdered roots of *Valeriana edulis* ssp *procera* was donated by Laboratorios Mixim, Mexico. The dry extract from roots of *Valeriana officinalis* were acquired from Amax NutraSource Inc., USA. Both valerian species were used as sedative plants references.

Preparation of the extracts

The air-dried and powdered plant materials (100 to 300 g) were successively extracted with hexane (3x2 L), dichloromethane (3x2 L) and methanol (3x2 L) by maceration at room temperature (22 ± 2 °C) for one day period. The extracts were filtered by gravity and the solvents were evaporated in vacuum. For seeds of *Casimiroa edulis*, a hydroalcoholic extract was prepared with 400 g of dried seeds and 2 L of a mixture of ethanol-water (7:3) for a 3 days period. The commercial powdered roots of *Valeriana edulis* ssp *procera* (100 g) and *Valeriana officinalis* (100 g) were extracted with 500 mL of a mixture of ethanol-water (7:3) for a 3 days period. The hydroalcoholic extracts were filtered by gravity and concentrated through stem air current at room temperature (22 ± 2 °C).

Chemicals and Drugs

Diphenhydramine and Tween 80 were purchased from Sigma Co. (Sigma St. Louis, MO). Absolute ethanol was of analytical grade and was purchased from Mallinckrodt Baker (Mexico). The other reagents were of analytical grade.

Animals

All experiments were performed on adult male ICR mice (25-34 g; Centro UNAM-Harlan, Harlan Mexico, S.A. de C.V.). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) adopted in our laboratory, and in compliance with international rules for care and use of laboratory animals. The experimental groups consisted of six animals. They were maintained at constant room temperature (22° ± 1 °C) and submitted to12 h light/dark cycle with free access to food and water.

Procedure

Diphenhydramine and hydroalcoholic extracts were dissolved in saline solution (0.9 %). All other extracts were suspended in 0.5 % Tween 80 in saline solution (0.9 %). The solutions and the suspensions were freshly prepared each time and intraperitoneally injected in a volume of 0.1 mL/10 g body weight. Control animals received the same volume of vehicle (0.5 % Tween 80 in saline or saline solution only). The diphenhydramine (used as sedative reference drug) and extracts were administered 30 minutes before testing at different doses.

The apparatus consisted of a glass cylinder (30 cm in height, 11 cm in diameter, with wall of 3 mm) set in a vertical position. The cylinder was placed over a flat surface covered with a filter paper in a room with constant lighting and isolated from external noise. An individual naïve mouse was put on the paper covered floor of the glass cylinder; the number of rearings performed over a 5 minutes period was recorded. The inner side of the apparatus was cleaned with alcoholic solution and the paper was changed between each animal test session (Ugalde *et al.*, 2005; Hiller and Zetler, 1996). During

Table 1. Medicinal plants used in the Mexican Traditional Medicine as sleep aids.

Number	Scientific name	Family	Popular name	Part used	Voucher
1	*Casimiroa edulis* Llave & Lex	Rutaceae	Zapote blanco	Seeds	IMSSM15457
2	*Citrus limon* (Christm.) Swingle	Rutaceae	Limón	Peel and pericarp	Commercial sample, México.
3	*Citrus sinensis* (L.) Osbeck	Rutaceae	Naranja	Flowers and leaves	IMSSM15436 (Flowers) IMSSM15437(Leaves)
4	*Cymbopogon citratus* (D.C.) Stapf	Poaceae	Zacate limón	Leaves	IMSSM15435
5	*Galphimia glauca* Cav.	Malpingiaceae	Cola de zorro	Aerial part	XOLO670
6	*Ternstroemia pringlei* (Rose) Standl	Theaceae	Flor de tila		IMSSM15459
7	*Ternstroemia sylvatica* Schltdl. & Cham.	Theaceae	Flor de tila	Fruits	IMSSM15460
8	*Valeriana edulis* ssp *procera* (Kunth) Meyer	Valerianaceae	Valeriana	Roots	Commercial sample, México.
9	*Valeriana officinalis* L.	Valerianaceae	Valeriana	Roots	Commercial sample, USA

Table 2. Sedative ED_{50} values of some organic plant extracts using the exploratory cylinder test in mice.

Specie	Part of the plant	Extract	ED_{50} ± SEM (mg/kg)
Cymbopogon citratus	Leaves	Hexane	27.71 ± 2.9
		Dichloromethane	77.11 ± 15.0
		Methanol	NA
Citrus limon	Peel	Hexane	NA
		Dichloromethane	NA
		Methanol	355.70 ± 71.5
Citrus sinensis	Flowers	Hexane	NA
		Dichloromethane	NA
		Methanol	47.04 ± 12.0
Citrus sinensis	Leaves	Hexane	400.21 ± 59.4
		Dichloromethane	NA
		Methanol	38.48 ± 8.0
Galphimia glauca	Aerial part	Hexane	76.85 ± 20.9
		Dichloromethane	NA
		Methanol	22.06 ± 5.6
Ternstroemia pringlei	Fruits	Hexane	558.00 ± 46.59
		Dichloromethane	631.00 ± 181.4
		Methanol	319.00 ± 49.62
Ternstroemia sylvatica	Fruits	Hexane	752.10 ± 208.44
		Dichloromethane	743.10 ± 208.34
		Methanol	61.88 ± 6.42
Casimiroa edulis	Seeds	Ethanol-water	802.80 ± 165.77
Valeriana edulis ssp *procera*	Roots	Ethanol-water	182.62 ± 11.62
Valeriana officinalis	Roots	Ethanol-water	452.01 ± 47.45
Diphenhydramine			17.06 ± 2.04

NA: not active until 1600 mg/kg

observation, the experimenter stood next to the apparatus, always in the same place. The observations were made without prior knowledge of the experimental conditions applied to the animal. Reduced exploratory rearing showed by naïve mice after placement in an unfamiliar environment reveals a sedative effect (Ugalde *et al.*, 2005; Hiller and Zetler, 1996; Oliva *et al.*, 2004).

Dose-Response curves were constructed for each extract using five different levels of doses. The dose that produced 50 % of sedation (ED_{50}, 50 % of reduction in the rears number with respect to control group) and its SEM was calculated using standard linear regression analysis of the log dose-response (Tallarida, 2000).

RESULTS AND DISCUSSION

Intraperitoneal administration of the organic and hydroalcoholic extracts of the species tested resulted in a dose-dependent decrease of the number of rearings in the exploratory cylinder model (Fig. 1). The purpose of evaluating extracts with different polarity was to locate the extract with the major activity of each plant to continue its study to identify the active compounds by a bio-guided fractionation. The most active extract was methanol extract of *Galphimia glauca* (ED_{50} = 22.06 ± 5.6 mg/kg), followed by hexane extract of *Cymbopogon citratus* (ED_{50} = 27.01 ± 2.9 mg/kg), methanol extract of leaves of *Citrus sinensis* (ED_{50} = 38.48 ± 8.0 mg/kg), methanol extract of flowers of *Citrus sinensis* (ED_{50} = 47.04 ± 12.0 mg/kg), methanol extract of *Ternstroemia sylvatica* (ED_{50} = 61.88 ± 6.42 mg/kg), dichloromethane extract of *Cymbopogon citratus* (ED_{50} = 77.11 ± 15.0 mg/kg). Other extracts showed ED_{50} values over 100 mg/kg (Table 2), whereas the hexane and dichloromethane extracts of *Citrus limon* peel, the hexane and dichloromethane extract of flowers and dichloromethane extract of leaves of *Citrus sinensis*, methanol extract of *Cymbopogon citratus* and dichloromethane extract of *Galphimia glauca* were inactive. On other hand, hydroalcoholic extract of Mexican valerian (*Valeriana edulis ssp procera*) showed a more potent sedative effect (ED_{50} = 182.62 ± 11.62 mg/kg) than *Valeriana officinalis* hydroalcoholic extract (ED_{50} = 452.01 ± 47.45 mg/kg) in the exploratory cylinder model. Diphenhydramine showed an ED_{50} value of 17.06 ± 2.04 mg/kg. This drug was used as sedative reference drug considering that both diphenhydramine and medicinal plants are used as sleep aids in a similar way. In addition the ED_{50} sedative values in the exploratory cylinder model for this drug is within of the range of those ED_{50} sedative values showed by the vegetal extracts.

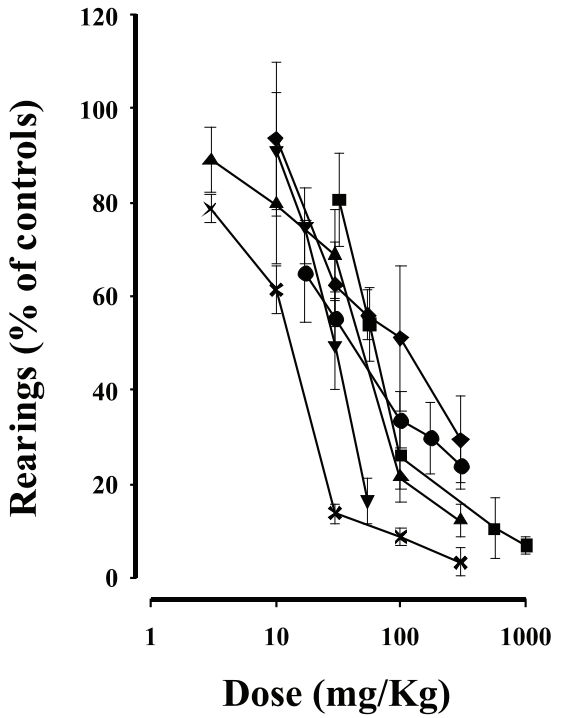

Figure 1. Dose–response curves plotting % of rearing with respect to its control represented on the y-axis for the sedative effect of methanol extracts of flowers of *C. sinensis* (●), fruits of *T. sylvatica* (■), leaves of *C. sinensis* (▲), aereal parts of *G. glauca* (×) and hexane (▼) and dichloromethane extracts (♦) of *C. citratus*. Values are expressed as mean ± S.E.M. of 6 experiments. Doses (mg/kg) are represented in a logarithmic scale on the x-axis.

The validity of the exploratory cylinder model for testing sedative drugs has been well established (Rolland *et al.*, 1991; Hiller and Zetler, 1996; Ugalde *et al.*, 2005; Oliva *et al.*, 2004; Balderas *et al.*, 2008). In this model the rearings number decrease with an increase of the doses of sedative drugs, and allows the construction of doses-res-ponse curves and the calculus of effective doses for individual drugs (Ugalde *et al.*, 2005).

In a previous work, Tortoriello and Lozoya (1992) describe the sedative effect for *Galphimia glauca* methanol extract based on the prolongation of sleep time induced by pentobarbital. This effect has been attributed to Nor-*seco*-triterpenoid Galphimine B, isolated from methanol extract (Tortoriello and Ortega, 1993). In the present work, both methanol and hexane extracts showed sedative effect in the exploratory cylinder model, suggesting that *Galphimia glauca* contains others non-polar sedative compounds in the hexane extract in addition to Galphimine B, that result interesting to identify.

The activity of *Cymbopogon citratus* on central nervous system (CNS) has already been investigated; the antinociceptive effect has been reported for essential oil and for the infusion prepared from fresh leaves of *Cymbopogon citratus* (Viana *et al.*, 2000; Lorenzetti *et al.*, 1991); Carlini and coworkers did not observe effect on the motor activity and rota-rod tests in rodents for the infusion of *Cymbopogon citratus* (Carlini *et al.*, 1986) and a double blind study with 50 health voluntaries indicated that the infusion did not have anxiolytic-like activity (Leite *et al.*, 1986). In contrast, in the present work a clear sedative effect was observed for hexane extract of this species, suggesting that the active compounds are of nonpolar type including the essential oil. The latter is supported for the low activity of the dichloromethane extract and the absence of sedative activity for the methanol extract. *Cymbopogon citratus* has high content of citral, a natural mixture of two isomeric aldehydes, neral and geranial (Leite *et al.*, 1986), therefore it is not possible to discard the participation of these components in the sedative activity of hexane extract, because the intraperitoneal administration of citral (100 mg/kg) was able to decrease the rearings in the open field test in a previous work (Carlini *et al.*, 1986) and recently has been reported also that intraperitoneal administration of the essential oil obtained from this medicinal plant in mice induces sedative effect (Blanco *et al.*, 2009).

It has been reported the enhancement of sleeping time induced by sodium pentobarbital, the anxiolytic activity in elevated plus maze model and anticonvulsant activity for essential oil from peel and hydroalcoholic extract of leaves from *Citrus aurantium* in rodents (Carvalho-Freitas and Costa, 2002; Pultrini *et al.*, 2006). In addition, in the present work the methanol extracts from leaves and flowers of *Citrus sinensis* showed sedative activity, suggesting that the active compounds should be polar compounds. Recently we identified the hesperidin as one of the active sedative compounds in flowers of *Citrus sinensis* (Guzmán-Gutiérrez and Navarrete, 2009); however, there are not additional phytochemical or pharmacological studies for flowers and leaves of this species. Studies in progress are running in our laboratory to investigate other active components of the flowers and leaves of methanol extract of *Citrus sinensis*.

The sedative, anxiolytic and anticonvulsant effects for leaves (Mora *et al.*, 2005; Molina-Hernández *et al.*, 2004) and the anticonvulsant activity for seeds (Garzón-de la Mora *et al.*, 1999) of *Casimiroa edulis* have already been reported. However the moderate sedative effect for the seeds of *Casimiroa edulis* is reported here for the first time.

The sedative effect observed for *Ternstroemia pringlei* and *Valeriana edulis* ssp *procera* are in agreement with previous works (Balderas *et al.*, 2008; Ugalde *et al.*, 2005). For *Ternstroemia sylvatica* it

has been reported its sedative activity in rat using simple and repeated intraperitoneal administration once a day for a week. However, these treatments failed to show anxiolytic activity in elevated plus maze model (Molina *et al.*, 1999). Commercial dry extract of *Valeriana officinalis* showed low sedative activity in the exploratory cylinder model (ED_{50} = 452.01 ± 47.45 mg/kg), even lower than the sedative effect showed for the commercial extract of *Valeriana edulis* ssp *procera* (ED_{50} = 182.62 ± 11.62 mg/kg). These species are reputed to possess a well demonstrated sedative effect. In this respect, some of the extracts tested here showed sedative activities at lower doses than those showed by *Valeriana* species, indicating a promissory sedative effect.

CONCLUSION

Using the exploratory cylinder test, it was possible to perform a quantitative comparison of the sedative effects of several medicinal plants commonly used in Mexico as sleep aids. In this way, plant species with promising sedative activity can be effectively screened and selected for further investigations, including the isolation of active compounds. In addition some species showed more potent sedative effect than valerian species, plants with a very well recognized sedative effect.

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REFERENCES

Balderas, J., Reza, V., Ugalde, M., Guzmán, L., Serrano, M., Aguilar, A., Navarrete, A. (2008) Pharmacodynamic interaction of the sedative effects of *Ternstroemia pringlei* (Rose) Standl. with six central nervous system depressant drugs in mice. *Journal of Ethnopharmacology* **119**: 47-52.

Benca, R. (2005) Diagnosis and treatment of chronic insomnia: A review. *Psychiatric services* **56**: 332-343.

Blanco, M.M., Costa, C.A.R.A., Freire, A.O., Santos, J.G., Costa, M. (2009) Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. *Phytomedicine* **16**: 265-270.

Carlini, E., Contar, J., Silva-Filho, A., Silveira-Filho, N., Frochtengarten, M., Bueno, O. (1986) Pharmacology of lemongrass (*Cymbopogon citratus* Staff). I. Effects of teas prepared from the leaves on laboratory animals. *Journal of Ethnopharmacology* **17**: 37-64.

Carvalho-Freitas M.I., Costa M. (2002) Anxiolytic and sedative effects of extracts and essential oil from *Citrus aurantium* L., *Biological and Pharmaceutical Bulletin* **25**: 1629-1633.

Edinger, J., Means, M. (2005) Cognitive-behavioral therapy for primary insomnia. *Clinical*

Psychology Review **25**: 539-558.

Garzón-de la Mora, P., García-López, P.M., García-Estrada, J., Navarro-Ruíz, A., Villanueva-Michel, T., Villarreal-de Puga, L.M., Casillas-Ochoa, J. (1999) *Casimiroa edulis* seed extracts show anticonvulsive properties in rats. *Journal of Ethnopharmacology* **68**: 275-282.

Gottesmann, C. (2002) GABA mechanisms and sleep. *Neuroscience* **111**: 231-239.

Guzmán-Gutiérrez, S.L., Navarrete A. (2009) Pharmacological exploration of the sedative mechanism of hesperidin identified as the active principle of *Citrus sinensis* flowers. *Planta Medica* **75**: 295-301.

Harvey, A. (2001) Insomnia: symptom or diagnosis? *Clinical Psychology Review* **21**: 1037-1059.

Hiller K.O., Zetler G. (1996) Neuropharmacological studies on ethanol extracts of *Valeriana officinalis* L.: Behavioral and anticonvulsant properties. *Phytotherapy Research* **10**: 145-151.

Instituto Nacional Indigenista. (1994) Flora medicinal indígena de México II. Biblioteca de la Medicina Tradicional Mexicana., México pp 594.

Leite, J., Seabra, M., Maluf, E., Assolant, K., Suchecki, D., Tufik, S., Klepacz, S., Calil, H., Carlini, E. (1986) Pharmacology of lemongrass (*Cymbopogon citratus* Staff). III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. *Journal of Ethnopharmacology* **17**: 75-83.

Lorenzetti, B., Souza, G., Sarti, S., Santos, D., Ferreira, S. (1991) Myrcene mimics the peripheral analgesic activity of lemongrass tea. *Journal of Ethnopharmacology* **34**: 43-48.

Molina, M., Contreras, C.M., Tellez-Alcantara, P., Rodriguez, F. (1999) Sedative actions of *Ternstroemia sylvatica* in the male rat. *Phytomedicine* **6**: 115-118.

Molina-Hernández, M., Tellez-Alcantara, N. P., Pérez, J., Olivera, J. I., Jaramillo, M. T. (2004) Anxiolytic-like actions of leaves of *Casimiroa edulis* (Rutaceae) in male Wistar rats. *Journal of Ethnopharmacology* **93**: 93-98.

Mora, S., Diaz-Veliz, G., Lungenstrass, H., García-González, M., Coto-Morales, T., Poletti, C., De Lima, T.C.M., Herrera-Ruiz, M., Tortoriello, J. (2005) Central nervous system of hydroalcoholic extract of *Casimiroa edulis* in rats and mice. *Journal of Ethnopharmacology* **97**: 191-197.

Nau, S.D., McCrae, C.S., Cook, K.G., Lichstein, K.L. (2005) Treatment of insomnia in older adults. *Clinical Psychology Review* **25**: 645-672.

Oliva, I., González-Trujano, M.E., Arrieta, J., Enciso-Rodríguez, R., Navarrete, A. (2004) Neuropharmacological profile of hydroalcohol extract of *Valeriana edulis* ssp. *procera* roots in mice. *Phytotherapy Research* **18**: 290-296.

Pultrini Ade.M., Galindo L.A., Costa M. (2006) Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. *Life Science* **78**: 1720-1725.

Rolland A., Fleurentin J., Lanhers M.C., Younos C., Misslin R., Mortier F., Pel, J.M. (1991) Behavioral effects of the American traditional plant *Eschscholzia californica*: Sedative and anxiolytic properties. *Planta Medica* **57**: 212-216.

Shigemoto Y., Shinomiya K., Mio M., Azuma N., Kamei C. (2004) Effects of second-generation histamine H1 receptor antagonists on the sleep-wakefulness cycle in rats. *European Journal of Pharmacology* **494**: 161-165.

Tallarida, R.J., (2000) Drug synergism and dose-effect data analysis. Chapman and Hall/CRC., USA pp. 21-35.

Tortoriello, J., Romero, O. (1992) Plants used by Mexican Traditional Medicine with presumable sedative properties: An ethobotanical approach. *Archives of Medical Research* **23**: 111-116.

Tortoriello, J., Lozoya, X. (1992) Effect of *Galphimia glauca* methanolic extract on neurophar-

macological tests. *Planta Medica* **58**: 234-236.

Tortoriello, J., Ortega, A. (1993) Sedative effect of Galphimine B, a nor-seco-triterpenoid from *Galphimia glauca*. *Planta Medica* **59**: 398-400.

Ugalde M., Reza V., González-Trujano M.E., Avula B., Khan I.A., Navarrete A. (2005) Isobolographic analysis of the sedative interaction between six central nervous system depressant drugs and *Valeriana edulis* hydroalcoholic extract in mice. *Journal of Pharmacy and Pharmacology* **57**: 631-639.

Viana, G., Vale, T., Pinho, R., Matos, F. (2000) Antinociceptive effect of essential oil from *Cymbopogon citratus* in mice. *Journal of Ethnopharmacology* **70**: 323-327.

Wheatley, D. (2005). Medicinal plants for insomnia: A review of their pharmacology, efficacy and tolerability. *Journal of psychopharmacology* **19**: 414-421.