ARTÍCULO ORIGINAL

Obtention of dry extract from aqueous extracts of *Justicia pectoralis* Jacq. (tilo)

Obtención de extractos secos a partir de extractos acuosos de *Justicia pectoralis* (tilo)

PhD. Jorge Enrique Rodríguez Chanfrau, PhD. Orestes Dario López Hernández, MSc. Yanier Núñez Figueredo, Tech. Carlos Rodríguez Ferrada, Tech. Antonio Nogueira Mendoza

Center for Drug Research and Development. Havana, Cuba.

ABSTRACT

Introduction: *Justicia pectoralis* Jacq. (Acanthaceae), is a medicinal species native to the American tropics. In Cuba it is used in folk medicine in the treatment of nervous disorders. The Cuban Pharmaceutical Industry is working on the development of technological processes for the obtention of pharmaceutical quality raw materials from natural products.
Objectives: develop a technological process to obtain dry extract from aqueous extracts of *J. pectoralis.*
Methods: a process to obtain dry extract by spray drying from aqueous extract of *J. pectoralis* was developed. The influence of solid-liquid ratio and extraction time on coumarin content was evaluated by applying a response surface methodology (RSM). The drying process and later pharmacological evaluation of the dry powder were studied.
Results: results show that solid-liquid ratio was the statistically significant main effect, having a strong positive influence on coumarin extraction in our working conditions. Extraction time and interaction between both factors were not significant for regression models. On the other hand, evaluation of the influence of inert additives on drying showed that the best results were obtained with the use of 30 % soluble starch, which did not affect sedative activity.
Conclusions: a solid-liquid ratio of 1:20 and an extraction time of 15 minutes were suggested as optimum parameters for coumarin extraction.

Key words: aqueous extract, coumarin, *Justicia pectoralis* Jacq., response surface methodology, pharmacological evaluation, sedative activity, spray drying.

RESUMEN

Introducción: *Justicia pectoralis* Jacq. (Acanthaceae) es una especie medicinal nativa de la América tropical. En Cuba es usada en la medicina tradicional en el tratamiento de afecciones nerviosas. La industria farmacéutica cubana trabaja en el desarrollo de procesos tecnológicos para la obtención de materias primas de calidad farmacéutica a partir de productos naturales.
Objetivo: desarrollar un proceso tecnológico para la obtención de extractos secos a partir de extractos acuosos de *Justicia pectoralis.*
Métodos: se desarrolló un proceso para la obtención de extracto seco por *spray drying* a partir de extracto acuoso de *Justicia pectoralis*. La influencia que sobre el contenido de cumarina tenían la relación sólido-líquido y el tiempo de extracción fue estudiada aplicando un diseño superficie respuesta. Se estudió el proceso de secado y la posterior evaluación farmacológica del polvo seco.
Resultados: estos mostraron que la relación sólido-líquido tenía una fuerte influencia estadísticamente significativa sobre la extracción de la cumarina en las condiciones de trabajo experimentadas. El tiempo de extracción y la interacción entre ambos factores no fueron significativos para el modelo de regresión estudiado. Por otro lado, la evaluación de la influencia de aditivos inertes en el proceso de secado mostró que los mejores rendimientos se alcanzaban cuando se empleaba almidón soluble 30 %, y no se afectaba la actividad sedante del producto.
Conclusiones: una relación sólido-líquido de 1:20 y un tiempo de extracción de 15 min resultaron las condiciones óptimas para la extracción de cumarina.

Palabras clave: extracto acuoso, cumarina, *Justicia pectoralis* Jacq., metodología superficie respuesta, evaluación farmacológica, actividad sedante, *spray drying.*

INTRODUCTION

Illnesses related to the Central Nervous System are a health problem in the world. Natural products have been used for the treatment and control of illnesses related to this pathology.

Justicia pectoralis Jacq. (Acanthaceae), known as Tilo, is a medicinal species native to the American tropics. It is used in folk medicine for its curative properties. In Cuba it is used in the treatment of alterations of the nervous system.[1] Various studies have revealed the presence of betaine, coumarin, 7-hydroxycoumarin, flavonoids (apigenin, swertisin and swertiajaponin), lignans and more than 15 amino acids.[2-4]

Coumarins belong to a group of compounds known as benzopyrones, which is the main constituent responsible for the aromatic fragrance of dried leaves of *Justicia pectoralis*.[5] Pharmacological activities of coumarin, for example anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, sedative and anticarcinogenic activities have been described.[4,6,7]

Nowadays, the Cuban Pharmaceutical Industry is working on the development of technological processes for the obtention of pharmaceutical quality raw materials from natural products. Spray drying is a simple and effective method to obtain dry powder

with attractive physical characteristics, excellent flow and packing properties that greatly facilitate handling and transport.[8,9]

In Cuba, the established process for *Justicia pectoralis* extract obtention is hydroalcoholic repercolation.[10] This procedure requires a lengthy extraction time (4 days) and a long ethanol evaporation time. On the other hand, drying studies carried out by *Rodriguez et al*, have demonstrated loss by adhesion of the product to the walls of the chamber during the drying process, resulting in poor product recovery.[11]

A process to obtain aqueous extract of *Justicia pectoralis* was developed. Extraction temperature was 100 ºC. The influence of solid-liquid ratio and extraction time on coumarin content was evaluated by applying response surface methodology (RSM). The drying process and later pharmacological evaluation of the dry powder was studied.

METHODS

Plant material

The sample of *Justicia pectoralis* Jacq. (var. pectoralis) was collected at Dr. Juan Tomás Roig Medicinal Plants Experimental Station–in Artemisa, Cuba. Herbarium specimens, voucher ROIG 4636, have been deposited in herbaria at the Experimental Station.

The aerial parts of *J. pectoralis* were washed with H_2O and 2 % sodium hypochlorite solution, and were dried at 45 ºC. After drying, the material obtained was stored in plastic bags until usage. In parallel, quality control was carried out according to the method described.[12]

Establishment of the technological process

Experimental design

Response Surface Methodology (RSM) with a 3^2 central composed experimental design (Statgraphics plus 5.1, USA) was applied to evaluate the effects of extraction time (ET) and solid-liquid ratio (SLR) on coumarin content (%). The coded independent variables used are listed in Table 1. The levels of independent parameters were based on preliminary experimental results.

Table 1. Coded levels of independent variables used in the response surface methodology (RSM) design

Independent variables	Coded level		
	low	medium	high
Extraction time (min)	15	67.,5	120
Solid liquid ratio (m/v)	1:5	1:12.5	1:20

Drying process

The preliminary study showed loss by adhesion of the product to the walls of the chamber during the drying process, making it necessary to employ inert additives. The inert additives used in our study were 10 DE Maltodextrin (Arancia-cpc, Mexico) and Soluble Starch (Riedel de Haën, Germany) at concentrations between 10 and 30 % versus the dry weight of liquid extract content.

The carried solution was prepared by dissolving the additive in 1000 mL of aqueous extract under constant stirring before spray drying. The aqueous extracts were spray dried in a Büchi B 191 model spray dryer (Switzerland). Inlet air temperature (140 °C) and outlet air temperature (80 °C) were carried out according to the method described by *Rodríguez et al*.[11] The product was fed into the spray dryer at room temperature 600 L/h: the rate was varied to regulate exit air temperature at the desired value. After cooling to room temperature, the powder was placed in plastic bags and stored until analysis.

Drying efficiency was assessed by determining the total powder content obtained in the process and comparing it with the theoretical powder content that can be obtained. Three batches (5 liter scale) with the best inert additive were prepared and drying efficiency was determined.

Aqueous extract and drying powder analysis

Coumarin content in the aqueous extract was determined by HPLC using an Aluspher® 100 (RP-select B 5 mm) pre-column and a Lichrospher® 100, RP 18 column (250 x 4 mm, 5 µm). The mobile phase used was methanol-water (60:40 v/v). Chromatography conditions were mobile phase flow 1 ml/min, UV detector λ= 274 nm, and injection volume 20 µL.

Coumarin content in the drying powder was determined according to the method described by *Rodríguez et al*.[13]

Dry matter content, pH and organoleptic characteristics in the aqueous extract were determined according to NRSP with some modifications.[14]

Pharmacological studies

Our interest was to know whether the technological process developed affected the sedative activity of the product. A pharmacological evaluation (Open field activity method) was conducted of samples obtained from the inert additives study. Later, a pharmacological evaluation was carried out of samples obtained in 5 liter scale.

Animals

Male albino mice (Swiss, 1822 g) in thiopental-induced sleep, open field activity and aggressive behavior test. All animals were housed in groups of five under standard laboratory conditions of temperature, humidity and lighting (12:12-h light/dark). Animals had free access to food and water, except during the experiment. They were deprived of food but not of water 6 h before drug administration. Each group consisted of ten animals. All experiments were carried out between 8:00 am to 11:00 am in accordance with the Institutional Animal Ethical Committee which approved the study. Animal care was in conformity with Canadian Council for Animal Care guidelines.

Evaluation of the influence on pharmacological activity

In the inert additives study for the evaluation of their influence on pharmacological activity, animals were divided into four groups: Distilled water–treated group (NC), Spray dried hydroalcoholic extract (20 mg/kg)-treated group (PC) obtained according to *Rodriguez et al*.,[11] Spray dried aqueous extract without inert additives (20 mg/kg)-treated group (TG), and Spray dried aqueous extract with inert additives (20 mg/kg)-treated group (TGIA).

Pharmacological evaluation of samples obtained to the scale of 5 liters

Animals were divided into five groups: Distilled water–treated group (NC), Diazepam (1 mg/kg)-treated group (PC), and Spray dried aqueous extract with inert additives (5, 20 and 80 mg/kg)-treated groups.

Open field activity

Open field activity was carried out according to a previously reported method with some modifications.[15,16] Thirty minutes after administration of vehicle or test compound a mouse was placed in the centre of a round open field 30 cm in diameter and 25 cm in height, and open field activity was measured during 6 minutes, recording how many times the animal stayed in the centre of the cage and the number of risings.

Aggressive behavior

This study was conducted according to the method described by *Pinna et al*.[17] A group of animals were isolated in individual cages during six weeks, while another group remained together. Thirty minutes after administration of vehicle or test compound, aggressive behavior was evaluated by the introduction of an intruder mouse into the isolated mice's home cage. Aggressive activity (biting attacks and wrestling) by isolated mice was measured as total fighting time during a 20 min period.

Thiopental-induced sleep

The Thiopental-induced sleep study was conducted according to the method described by *Fernandez et al*.[18] Thiopental Sodium (50 mg/kg) was injected intraperitoneally 30 min after administration of distilled water or test compound. An animal was placed on its back on a warmed (35 ºC) pad. The number of sleeping animals and the duration of loss of righting reflex were recorded. The time elapsed from loss of righting reflex until a mouse regained its righting reflex was measured.

Statistical analysis

Experimental design, data analysis and optimization procedure were performed using Statgraphic plus 5.1. All pharmacologically obtained results were expressed as mean ± standard deviation (SD) and assessed by analysis of variance (ANOVA) followed by Duncan's test. Results were considered significant when $p < 0.05$.

RESULTS

Technological studies

The dried plant material complies with the quality specifications established by the method of analysis.[12]

Maximum coumarin content (4.3 %) was recorded under the following experimental conditions: solid-liquid ratio 1:20 and extraction time 15 min. Analysis of variance (ANOVA) was used to evaluate the significance of coefficients in the models. Table 2 shows the regression coefficient values of equation and statistical parameters obtained from ANOVA. The p values were used to evaluate the importance of each coefficient and the interactions among the variables. To obtain a simple and yet realistic model, insignificant terms ($p > 0.05$) are eliminated from model through a backward elimination process. The statistical parameters obtained from the ANOVA for the reduced models are given in Table 2. Figure 1 shows the Pareto chart for the response variable.

Table 2. Regression coefficients and statistical parameters obtained from ANOVA (before and after backward elimination)

Parameter	Regression coefficient	Standar error	*t* ratio	p value	Adjusted R^2
X_1	0.134	0.443	30.61	0.002	0.8835
X_2	- 0.002	0.443	0.35	0.578	
X_1 [a]	0.134	0.422	33.76	0.000	0.9037

[a]: only significant coefficients with $p < 0.05$ are included.

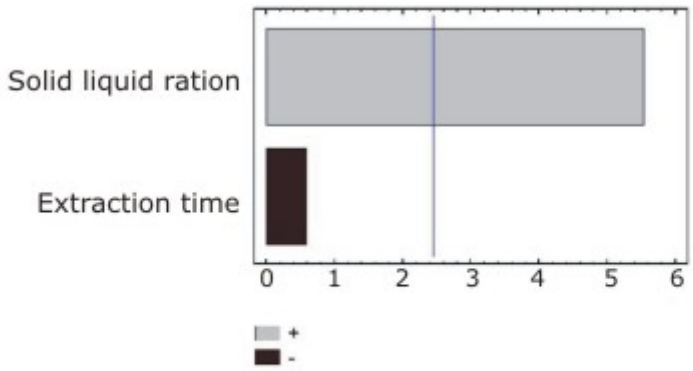
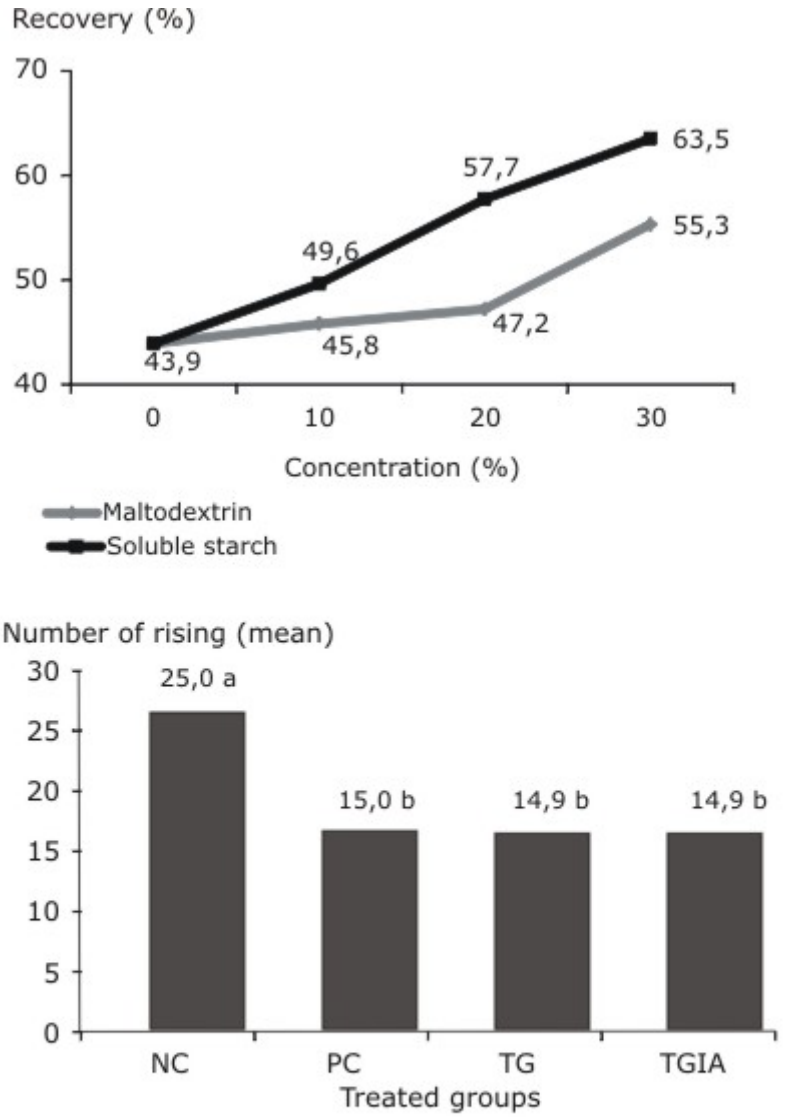


Fig. 1. Pareto chart for the response variables considered in this study.

A transparent liquid of brownish gray color and characteristic fragrance was obtained. Dry matter content in the extract was 3.27 ± 1.11 and pH was 5.41 ± 1.14.

Figure 2a shows the results of the influence of both inert additives on the drying of the aqueous extract of *Justicia pectoralis*. The best results were obtained with the use

of the 30 % soluble starch (drying efficiency above 65 % considered appropriate for the working scale). On the other hand, no loss by adhesion of the product to the walls of the chamber during the drying process was observed, which favors product recovery.



a: influence of the use of 10 DE maltodextrin and soluble starch on drying of the aqueous extract of *Justicia pectoralis*; b: result of the evaluation of the influence of the inert additive on pharmacological activity.

Fig. 2. Result of the evaluation of the inert additive.

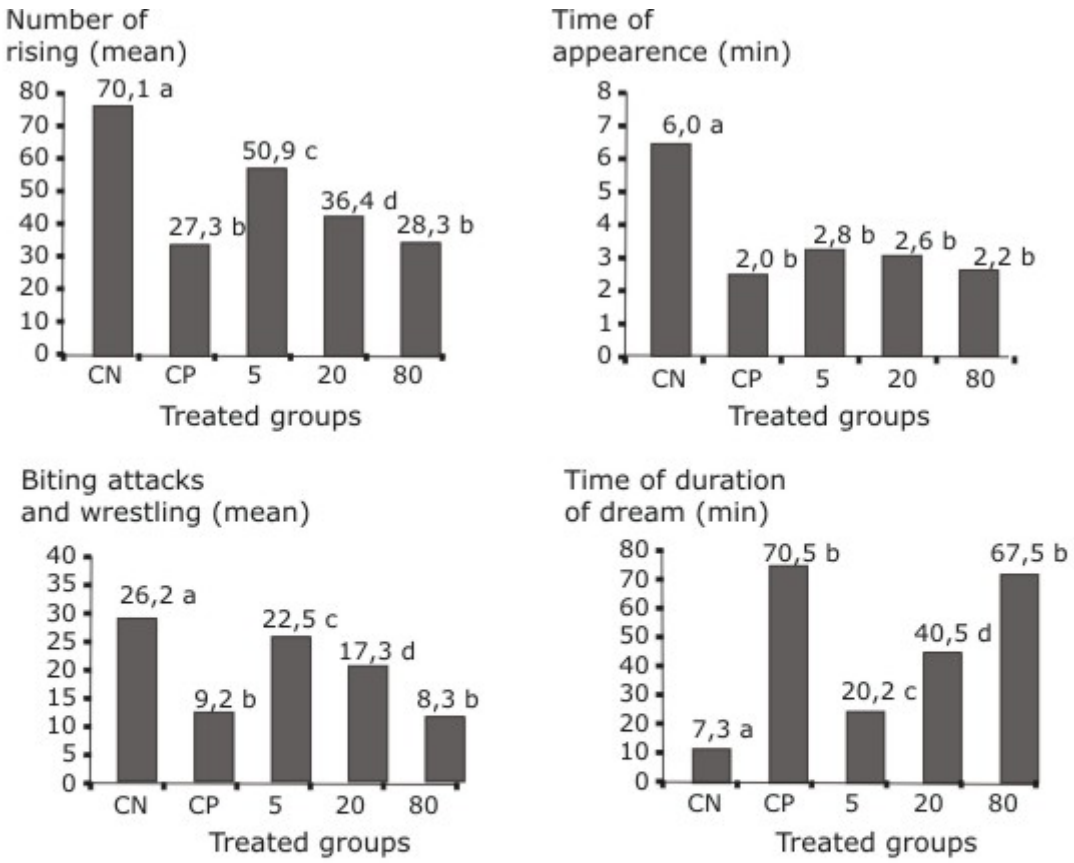
A fine and amorphous powder of brown color was obtained. Coumarin content was more than 7.0 mg/g of dried powder. These results are similar to those reported by *Rodriguez et al*.[18] in studies of drying of hydroalcoholic extracts of *Justicia pectoralis* by spray drying. Results of analysis of batches with 30 % soluble starch and an exit air temperature of 80 ºC were that those conditions were optimal to obtain dry

powder from aqueous extract of *Justicia pectoralis* with a recovery greater than 70 %, considered appropriate for the working scale.

Pharmacological studies

Evaluation of the influence of inert additives on pharmacological activity (Open field activity method) showed no significant differences ($p < 0.05$) between the spray dried hydroalcoholic extract treated group (PC), the spray dried aqueous extract without inert additives treated group, and the spray dried aqueous extract with 30 % soluble starch treated group (Fig. 2b).

In the pharmacological evaluation of samples obtained to the scale of 5 liters, the open field activity study (Fig. 3a) found that dried extract (5, 20 and 80 mg/kg, po.) reduced spontaneous locomotors activity and rearing in a dose-dependent manner during the observation period. Figure 3b shows the effects of test compound on aggressive behavior in isolated mice. Test compound reduces aggressive behavior in a dose-dependent manner.



a: results of open field activity in mice treated; b: Results of aggressive study in mice treated and Effect of dried extract on Thiopental-induced sleep in mice; c: time of appearance of induced dream; d: time of duration of sleep. Similar letter not significant for $p < 0.05$).

Fig. 3. Pharmacological evaluation of samples obtained to the scale of 5 L.

The time of appearance of induced sleep in animals treated with the different doses of dry extract was significantly shorter ($p < 0.05$) than that obtained ~~f~~ in the negative control group (Fig. 3c). All test compound doses increased the number of sleeping animals compared with the control. Doses of 20 and 80 mg/kg caused sleep in all animals. Test compound 80 mg/kg and diazepam 1 mg/kg prolonged thiopental-induced sleep in a similar manner (Fig. 3d).

DISCUSSION

Experimental design

The process of optimization by RSM is advantageous because this method saves time, space and raw materials. Application of this method allowed us to study the main and possible effects of extraction time and solid-liquid ratio on coumarin content.

Results show that the different indicators of the process did not exhibit the same pattern. Solid-liquid ratio showed the statistically significant main effect, having a strong positive influence on coumarin extraction in our working conditions. Extraction time and the interaction between the two factors were not significant for regression models.

With respect to numerical optimization, a solid-liquid ratio of 1:20 and an extraction time of 15 minutes were suggested as optimum parameters for coumarin extraction from *J. pectoralis*. Suitability of the model equation for predicting optimum response values was tested under these~~s~~ conditions and was validated experimentally. A mean value of 4.32 % of coumarin, obtained from real experiments (4.35 % value predicted), demonstrated the validity of the RSM model, indicating that the model was adequate for the extraction process.

Evaluation of the influence of inert additives on drying

The presence of water in solid-state systems has a significant impact on the stability of drugs not only by causing hydrolysis, but also because it affects other properties such as plasticity and ductility, film forming ability, and stickiness.[19]

Spray drying is a simple and effective method to obtain dry powder. The presence of some amorphous compounds~~,~~ with high contents of water causes adhesion of the product to the walls of the drying chamber during the process, resulting in poor product recovery. This situation is solved using excipients (inert additives) that absorb water improving the drying process.[19] The amorphous state is the most reactive, and can be a source of stability problems~~,~~ upon process scale-up. Each product has a different glass transition temperature (Tg), resulting in differences in physical and chemical stability. One of the most important parameters characterizing an amorphous form is its low glass transition temperature, which is correlated with properties such as plasticity and ductility, film forming ability, and stickiness. Therefore, inert additives are contributory to drying, favoring recovery by increasing the glass transition temperature. It has been reported that when individual indexes of drying (IID) are greater than 1 (therefore a high Tg value), the products are dried without any difficulty. However, when individual indexes of drying are smaller than 1, the products are dried with difficulty, sticking to the inner surfaces of the dryer, which results in poor product recovery.[20]

The inert additives used in our studies have individual indexes of drying greater than 1 and a high Tg value (Tg: 160 and IID: 1.6 for 10 DE Maltodextrin, Tg: 243 and IID: 2.4 for Soluble Starch), appropriate to improve the drying process. However, the glass transition temperature (Tg) of the aqueous extract of *Justicia pectoralis* was 59.6 ºC and the sticking temperature (Ta) was 79.7 ºC. Additionally, it was estimated that the glass transition temperature and the sticking temperature of the aqueous extract of *Justicia pectoralis* with soluble starch inert additive were 72.1 ºC and 92.1 ºC, respectively.[21] This result is important because the fixed exit air temperature (80 °C) in the technological process, which is smaller than the sticking temperature of the aqueous extract/inert additive mixture (92.1 ºC), prevents sticking during the drying process, increasing product recovery. Results show that the technological process developed was suitable to obtain a pharmaceutical quality raw material.

Pharmacological studies

Results on the influence of inert additives on pharmacological activity suggest that the addition of 30 % soluble starch does not affect the sedative activity of *J. pectoralis*.

Pharmacological evaluation of samples obtained to the scale of 5 liters

The open field activity study of samples obtained to the scale of 5 L showed that dried extract reduced spontaneous locomotors activity and rearing in a dose-dependent manner during the observation period. Rodents usually show exploratory behavior when they are placed in a new environment. However, if the animals are pre-treated with depressant drugs, locomotive activity is decreased. Doses of 80 mg/kg of test compound showed similar behavior to diazepam 1 mg/kg (standard anxiolytic drugs). This result is typical of sedative drugs.

A result similar to that of the open field test between 80 mg/kg of dried powder and diazepam 1 mg/kg doses was obtained for aggressive behavior. This behavior was reported by *Valzelli* as a classic sign of depressed central nervous system.[22]

Dried extract obtained from aqueous extract of *J. pectoralis* as well as diazepam, a standard reference drug, increased the number of sleeping animals and prolonged thiopental-induced sleep time in mice.

Social isolation induces aggressive behavior in several strains of mice. Isolation-induced aggression is considered to be useful as an animal model for assessing inhibitory activity of the central nervous system. Different neurotransmitters such as serotonin, noradrenalin, dopamine and gamma aminobutyric acid (GABA) are considered to be involved in mediating aggressive behavior, with conflicting results on brain neurotransmitter metabolism.[23,24] In a recent study (not published) we were unable to demonstrate anxiolytic-like activity in the test compound using the elevated plus-maze test in mice. Therefore, the sedative effect of the powder extract obtained from aqueous extracts of *Justicia pectoralis* Jacq. is not mediated via the GABA or Glycine systems.

Our results show anti-aggressive behavior in powder extract-treated mice. This result can be mediated by inhibitory effects on brain biogenic amines action or excitatory neurotransmitter release, and suggests an inhibitory effect on the central nervous system. This suggestion was strengthened by the increase in sleeping time in the test-compound treated groups over that in the control group in the thiopental-induced sleep model.

In summary, it was found that solid-liquid ratio was the most significant factor affecting coumarin extraction. On the other hand, evaluation of the influence of inert additives on drying shows that the best results were obtained with the use of 30 % soluble starch, not affecting the sedative activity of *J. pectoralis*. Results show that the technological process developed was suitable to obtain a pharmaceutical quality raw material from aqueous extract of *J. pectoralis* by spray drying.

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Jorge Enrique Rodríguez Chanfrau. Centro de Investigaciones y Desarrollo de Medicamentos. Ave 26 No. 1605. Nuevo Vedado. La Habana. CP 10600. Cuba. E-mail: jorge.rodriguez@infomed.sld.cu