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Pharmacological Activity and Phytochemical Studies of *Erythrina crista-galli* Extracts

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Abstract

Erythrina crista-galli is a tree of Fabaceae family, native of the Rio de la Plata region. Its aerial parts are used in traditional medicine because of its sedative properties. In order to validate this traditional use aqueous and organic extracts of the leaves were tested by the Hippocratic Screening Test, Spontaneous Locomotors Activity and Potentiation of Pentobarbital Sleeping Time Test. Two extracts from all essayed resulted in a statistically significant depression in CNS. Bio-guided fractionation of the extracts using the Motility Test in mice was performed by different chromatographic techniques. Two purified fractions, both free of alkaloids, showed preliminary sedative properties. The chemical structure determination of their active constituents and their whole pharmacological profile are now in progress.

Keywords: *Erythrina crista-galli*; CNS; sedative activity.

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Introduction

Erythrina crista-galli L. (common name: *ceibo*) - an indigenous tree from the Río de la Plata region - is the National Flower of Uruguay and Argentina. Pioneering work by Deulofeu (1959) described for the first time isoquinoline tetracyclic spiro-alkaloids with curarizing properties in seeds and bark (Henry, 1949; Bruneton, 1993). Ethnobotanical uses of the plant are related to the sedative properties and analgesic action of the leaves in local treatments of injuries either as an infusion or cataplasm (González et al., 1941; Priore et al., 1989; Lahitte et al., 1998). The natives of Uruguay, the so-called *Charrúas*, probably chewed young leaves and flowers of *ceibo* because of the psychotropic action (Anton, 1998).

Since these pharmacological properties could not be directly adscribed to a curare-like action, and the alkaloid content of the leaves is very low, further pharmacological and phytochemical profiles of the *ceibo* leaves are required. In the present study, we report our results on the validation studies of the traditional use of *E. crista-galli* leaves, after a bio-guided fractionation of different extracts of the leaves as well as a preliminary characterization of their active constituents.

Experimental

Plant material.
Aerial parts of *Erythrina crista-galli* were collected in Maldonado Department, Uruguay by one of the authors (S. E.).

A voucher specimen (MVFQ 4194) is deposited in the Herbarium José Arechavaleta of the Botany Laboratory, Organic Chemistry Department, Facultad de Química Universidad de la República, Uruguay. Plant samples, were dried in a dark and fresh place.

Extraction and Fractionation Procedures.
Powdered dried aerial parts of *E. crista-galli* were separately extracted as follows: 415 g of plant material with 6.4 L of EtOH/water (7:3, v/v) at room temperature during a week, concentrated *in vacuo* and lyophilized (**E_1**); 500 g with 5 L of boiling purified water during 30 min, followed by lyophilization (**I_1**); 400 g with 4 L of dichloromethane at room temperature for 7 days, concentrated *in vacuo* (**E_2**); 200 g with 2 L of boiling 10% aq. solution of Na_2CO_3, pH 10, during 30 min, followed by neutralization with HCl, lyophilization and extraction with MeOH (**I_2**); 500 g with 5 L of 0.1N HCl/30% EtOH (4.2:0.8) at room temperature during 9 days, neutralized with Na_2CO_3 followed by exhaustive extraction with dichloromethane (**E_3**). **E_1** was subjected to a biphasic hydrolysis with 2N HCl/$CHCl_3$ (1:2) for 1 hour under reflux. The chloroform layer was concentrated *in vacuo* (**J**). All solvents were glass-distilled prior to use. Otherwise stated, bidistilled water is used.

Those extracts that showed a pharmacological CNS activity of interest by the Hippocratic Test were further bio-guided fractionated using the Motility Test.

The fractionation procedures included VLC (Silica gel 60) and column chromatography (Silica gel 60H), eluting with solvent mixts of increasing polarity containing *n*-hexane, dichloromethane, EtOAc and MeOH. Preparative TLC over silica-gel plates has been used with the following mobile phase: EtOAc/AcOH/HCOOH/water (10:1.1:1.1:2.6, in vol.).

Table 1- Results of the Hippocratic Screening Test

Extract	Dose (mg/kg, i.p.)	Indication of Depressive CNS activity	Indication of Analgesic activity	Curare-like effects
E_1	400	+	-	-
E_2	100	+	+	-
E_2	200	+	+	-
E_3	1	-	+	-
E_3	10	-	+	-
E_3	50	-	+	-
I_1	500	+	-	-
I_2	300	+	-	-
J	300	+	-	-

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Animals.
Males CD-1 mice (27±3 g) were used in all experiments, except for the Observational Screening Test with male Sprague-Dawley rats (250±50 g).

All animals came from our own breeding colony, and they were kept in a standard controlled environment according to the Guides of the National Research Council (1999) (temperature, humidity, light-dark cycle, noises, etc). They were allowed standard laboratory feed and purified water *ad libitum*. To avoid any influence of circadian rhythmus, all assays were performed at the same time of the day. Precautions were taken to ensure that the animals received no external stimuli.

Pharmacological Methods.
The activity of the *E. crista-galli* extracts on CNS was evaluated by a general observational test: Hippocratic Screening Test (Malone and Robichaud, 1962), Spontaneous Motor Activity (Boissier et al., 1972) and Potentiation of Pentobarbital Sleeping Time (De-Paris et al., 2000). All pharmacological protocols were submitted and approved by the Ethic Committee for Animal Experimentation of the Faculty of Chemistry, Universidad de la República, Uruguay. Animal manipulation was subjected to the Guides of the National Research Council (1999). Pain, distress and discomfort of the animals involved in the experiments were evaluated by the Morton and Griffiths advices (Morton and Griffiths, 1985).

Hippocratic Screening Test.
Treated groups of 10 male albino Sprague-Dawley rats were intraperitoneal (i.p.) injected with the extracts, while control groups received vehicle under the same conditions.

The extracts were vehiculized with a 5% solution of Tween 80 in saline and further tested with: $\mathbf{E_1}$ 400 mg/kg, $\mathbf{E_2}$ 100 and 200 mg/kg, $\mathbf{E_3}$ 1 mg/kg, 10 mg/kg and 50 mg/kg, $\mathbf{I_1}$ 500 mg/kg and $\mathbf{I_2}$ 300 mg/kg.

Motility Test.
Spontaneous locomotor activity was recorded using an activity cage (Letica) with automatic counting of animal movements across the bars on the cage floor. The animals, in groups of three, were treated with the extracts 30 min before they were placed in the activity cage for 10 min. Similar observations were recorded for the standard group (Diazepam 2 mg/kg) as well as for the control group (vehicle).

Table 2- Results of Motility Test experiments in mice.

Extract/Fraction	Dose (mg/kg)	% Decrease Spontaneous Locomotor Activity
$\mathbf{E_2}$	30 i.p.	No active
$\mathbf{F_1}$	30 i.p.	22
A	20 i.p.	44
B	20 i.p.	No active
$\mathbf{I_2}$	300 i.p.	36
$\mathbf{I_2}$	200 (orl)	26
J	300 i.p.	44
E	30 i.p.	77

Potentiation of Sodium Pentobarbital Sleeping Time Test.
Groups of CD-1 male mice were i.p. injected with *E. crista-galli* extracts. Control (saline) and standard (Diazepam 2 mg/kg) groups were similarly treated.Thirty min after i.p. injection, all groups received pentobarbital (40 mg/kg, i.p.). The latency of the loss of the righting reflex and the total sleeping time (time between the loss and the recovery of the righting reflex) were determined.

Statistical Analysis.
Each data set was tested for standard normal distribution with D'Agostino Test with $\alpha = 0.05$, and was analysed by

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matched Student's t-test. P values less than 0.05 were considered statistically significant.

Results

From dried powdered plant material several extracts were prepared. $\mathbf{E_1}$ (EtOH/water 7:3; yield 14.0%), $\mathbf{I_1}$ (infusion; yield 17.0%), $\mathbf{E_2}$ (dichloromethane; yield 6.9%), $\mathbf{E_3}$ (alkaloids enriched extract; yield 0.3%), $\mathbf{I_2}$ (methanolic extract of alkaline infusion; yield 12.0%). Upon a biphasic hydrolysis of $\mathbf{E_1}$, an aglicone enriched fraction **J** was obtained, in a yield of 14.0%.

The following extracts showed depressor activity in the CNS Hippocratic Screening Test: $\mathbf{E_2}$ at 200 and 100 mg/kg, $\mathbf{I_1}$ at 500 mg/kg, $\mathbf{I_2}$ at 300 mg/kg and **J** at 300 mg/kg (**Table 1**).

The alkaloids-enriched extract, $\mathbf{E_3}$, was tested in the Hippocratic Test. There was no evidence of i.p. curarizing activity at 1, 10 and 50 mg/kg. No signs of CNS depressor activity were shown. Signs suggesting an analgesic-anesthetic activity were observed, even at a dose of 1 mg/kg.

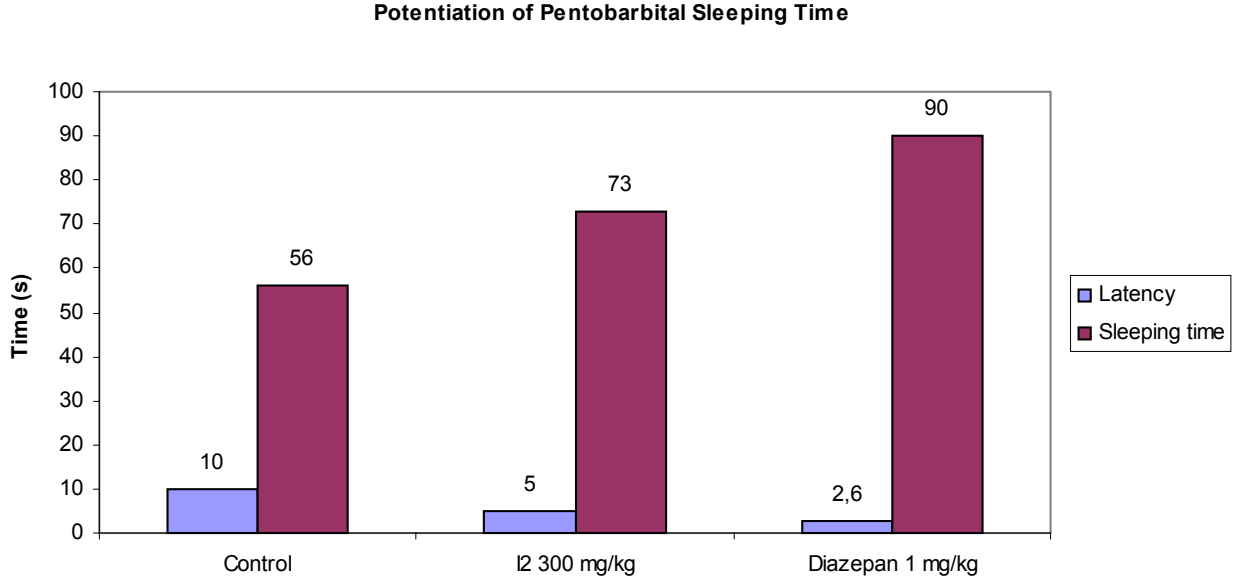

Figure 1. Effects of pretreatment with $\mathbf{I_2}$ (300 mg/kg, i/p), Diazepam 1 mg/kg, and saline (Control group) on Sleep Latency Time and Sleeping Time caused by pentobarbital (40 mg/kg) in mice. The results are reported in minutes.

The dichloromethane extract $\mathbf{E_2}$ did not show any chromatographic evidence of the occurrence of alkaloids, but of containing phenolic compounds as well as flavonoids. $\mathbf{E_2}$ showed CNS depressor activity and also indications of an analgesic-anesthetic activity. There was no evidence of curare-like activity. Animals were kept under observation for a week after administration of the extracts, and no death was recorded at any dose used.

$\mathbf{E_2}$ was tested in the Motility Test at a dose of 300 mg/kg (51% p=0.05 of depression in Spontaneous Locomotor Activity), while at 30 mg/kg there were no significant changes. As $\mathbf{E_2}$ exhibited a remarkable pharmacological activity, further studies were carried out. Therefore, $\mathbf{E_2}$ was fractionated through a VLC column using pure solvents of increasing polarity, yielding four fractions: $\mathbf{F_1}$ petroleum ether (PE), $\mathbf{F_2}$ ethyl ether, $\mathbf{F_3}$ ethyl acetate, and **F4** EtOAc/MeOH (7:3, in vol.). The PE fraction $\mathbf{F_1}$ (yield 0.2%) was the only fraction found to have CNS activity. A significant decrease of spontaneous locomotor activity was observed (22%; p= 0.05 at 30 mg/kg i.p. dose). On Hippocratic Screening analgesic activity was also detected. Neither alkaloids nor phenolics were detected by chromatographic analysis of this fraction. $\mathbf{F_1}$ was further fractionated by column chromatography (Silica gel 60H) leading to the following fractions: **A** (78% of $\mathbf{F_1}$) and **B** (22% of $\mathbf{F_1}$).

Fractions **A** and **B** were tested in the Motility Test at 20 mg/kg i.p. Fraction **A** gave a decrease of 44% p=0.004 in the Spontaneous Locomotor Activity, while Fraction **B** was inactive.

The chromatographic profiles of $\mathbf{I_2}$ and **J** looked very similar and have similar Rfs. There is no evidence of the occurrence of phenolics and/or alkaloids. They were tested in the Motility test at 300 mg/kg i.p., using saline as blank solution for $\mathbf{I_2}$ and 10% Tween 80 in saline for

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J. Both extracts were active, $\mathbf{I_2}$ gave a 36% reduction p=0.03 in the Spontaneous Locomotor Activity, while **J** produced a depletion of 44% p=0.006. Also, $\mathbf{I_2}$ was tested orally in the Motility Test at 200 mg/kg giving a 26% reduction p=0.008 (**Table 2**).

$\mathbf{I_2}$ was also i.p. tested in the Potentiation of Pentobarbital Sleeping Time Test at 300 mg/kg. The Latency Time before sleeping was significantly diminished (58%, p=0.002), while the Sleeping Time was enlarged (30%, p=0.00005) (**Fig. 1**).

$\mathbf{I_2}$ has been fractionated by preparative TLC using the following mobile phase: EtOAc/AcOH/HCOOH/water (10:1.1:1.1:2.6, in vol.). Two major fractions were obtained, **E** and **G**. **E** (yield 10% of $\mathbf{I_2}$) was tested in the Motility Test at dose of 30 mg/kg i.p. The fraction **E**, an almost pure fraction, produced a decrease of Spontaneous Locomotor Activity of 77% p=0.0015. Its chemical structure elucidation is now in progress.

Discussion and conclusions

Our results validate the use of *ceibo* leaves as a psychotropic agent. The Hippocratic Screening Test suggests a CNS depressor activity, whilst the tested analgesic-anesthesic action must be further confirmed using specific assays (Hot Plate Test, Mouse Writhing Assay, Formalin Test, etc). Both the Motility Test and the Potentiation Pentobarbital Sleeping Time Test gave additional evidence of the sedative action. The observed decrease of the locomotor activity after administration of extracts and fractions of *E. crista-galli* leaves suggests that CNS depressant effects, being confirmed by the results obtained in the Pentobarbital Induced Hypnosis. The $\mathbf{I_2}$ extract is CNS active when administered both orally and intraperitoneally.

The purified fractions **A** and **E** are both CNS active. The constituents of these fractions are being isolated and their structures are being elucidated. The latter are not alkaloids nor flavonoids.

Regarding the "curare-like" activity reported for the *E. crista-galli* alkaloids (Craig, 1981), experiments did not confirm it for the *ceibo* extracts. This is probably due to the low content of these alkaloids (0.3%) in the leaves.

These results were expected, since the known traditional use for treatment of injured skin would not be possible in the case of extracts with curare-like activity. During the Hippocratic Screening Test the extracts showed very low toxicity and no curarizing activity even though the extracts were administered intraperitoneally.

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References

-Anton D (1998) *El pueblo jaguar*; Piriguazu Ediciones; Montevideo, Uruguay.
-Boissier JR, Simon P, Zaczincka M, Fichelle J (1972) Etude psychopharmacologique expérimentale d'une nouvelle substance psychotrope, la 2-ethylamino-6-chloro-4-méthyl-4-phenyl-4H-3,1-benzoxazine. *Thérapie* **27**:325-338.
-Bruneton J (1993) *Pharmacognosie, Phytochimie, Plantes Medicinales*, pp 734, 2nd. Ed. Lavoisier; Paris, France.
-Craig L (1981) *Curare like effects.* In: Mansk RF, Holmes HL (Eds) *The Alkaloids Chemistry and Physiology*, Academic Press, New York, 265-293, U.S.A.
-Deulofeu V (1959) *The curarizing alkaloids of Erythrina species.* In: Bovet D, Bovet Nittland F, Marini Bettolo GB (Eds), *Curare and Curare-like agents.* Elsevier, Amsterdam,The Netherlands.
-De-Paris F, Neves G, Salgueiro JB, Quevedo J, Izquierdo I, Rates SMK (2000) Psychopharmacological screening of *Pfaffia glomerata* Spreng. (Amaranthaceae) in rodents. *J. Ethnopharmacol.* **73**: 261-269.
-González M, Lombardo A, Vallarino AJ (1941) *Plantas de la Medicina Vulgar del Uruguay*; pp 39, Talleres Gráficos Cerrito 580; Montevideo, Uruguay.
-Henry TA (1949) *The Plant Alkaloids.* pp 371. 4th Ed. J&A Churchil Ltd, 104 Gloucester Place, U.K.
-Institute of Laboratory Animal Resources, National Research Council (1996) *Guide for the Care and Use of Laboratory Animals.* National Academy Press, Washington, U.S.A.
-Lahitte HB, Hurrel JA, Belgrano MJ, Jankowski L, Haloua P, Mehltreter K (1998) *Plantas Medicinales Rioplatenses*, pp 160-167; Buenos Aires, Argentina.
-Malone MH, Robichaud RC (1962) A hippocratic screening for pure and crude drugs materials. *Lloydia* **25**: 320-332.
-Morton DB, Griffiths PHM (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* **116**: 431-436.
-Priore H, Carpiuc L, Alonso E, Bassagoda MJ (1989) Guía Taxonómica de Plantas que se comercializan como Medicinales en Uruguay. Primera Contribución. *Acta Farmacéutica Bonaerense* **8**: 187-198.
-Snedecor GW, Cochran WG (1977) *Métodos Estadísticos, Co.*; pp 123-156; Ed. Mexicana, Méjico.