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Anti-inflammatory evaluation and antioxidant potential of *Senna crotalarioides* and *Penstemon roseus*

[Evaluación anti-inflamatoria y potencial antioxidante de *Senna crotalarioides* y *Penstemon roseus*]

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Abstract
Chloroform, methanol and aqueous extracts of *Penstemon roseus* and *Senna crotalarioides* were investigated for their anti-inflammatory and antioxidant properties. The aqueous extract had no activity on TPA-induced ear edema, but the chloroform and methanol extracts of both plants caused a significant inhibition of the edema. The chloroform extracts showed activity on carrageenan-induced paw edema and mouse ear edema induced by multiple topical applications of TPA, and only *P. roseus* (dose of 100 mg/kg) exhibited anti-arthritic activity. The methanol extracts of *P. roseus* and *S. crotalarioides* had the most activity in the DPPH and reducing power tests. The LD_{50} values in mice of both chloroform extracts were considerate as slight acute toxic and higher than the doses used in this study.

Keywords: *Penstemon roseus, Senna crotalarioides*, anti-inflammatory, antioxidant, medicinal plants

Resumen
Las propiedades antiinflamatorias y antioxidantes de los extractos de cloroformo, metanol y agua de *Penstemon roseus* y *Senna crotalarioides* fueron investigadas. El extracto acuoso no tuvo actividad sobre el edema auricular inducido con TPA, pero el extracto de cloroformo y etanol de ambas plantas inhibieron significativamente el edema. El extracto de cloroformo mostró actividad sobre el edema plantar inducido con carragenina y el edema auricular en ratón inducido por múltiples aplicaciones de TPA, y solo *P. roseus* y *S. crotalarioides* tuvieron la mejor actividad en las pruebas de DPPH y poder reductor. Los valores de la DL_{50} en ratón de ambos extractos de cloroformo son considerados con ligera toxicidad aguda y mayores que las dosis empleadas en el estudio.

Palabras Clave: *Penstemon roseus*, *Senna crotalarioides*, anti-inflamatorio, antioxidante, plantas medicinales.

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INTRODUCTION

Penstemon roseus (Sweet.) G. Dom (Scrophulariaceae) is commonly known as jarritos, chulpa or cantaritos. It is a rounded herb up 40 to 60 cm tall with lanceolate leaves that are 3 to 9 cm long with very abundant red flowers; it grows in *quercus*, *pinus* and *abies* forests. In traditional medicine, this plant is used to heal infected wounds, insect bites and as an insecticide (Villavicencio et al., 2002).

Senna crotalarioides Kunth (Fabaceae) is a plant puberolent that grows in the Nuevo León and San Luis Potosi States of Mexico. In traditional medicine, it is used to treat different types of inflammation.

Is well known that oxygen and nitrogen reactive species are produced in all mammalian cells as the result of normal cellular metabolism, and these species play roles in normal physiological processes, in protection against pathogens, cellular signaling pathways and the regulation of vascular tone (Valko et al., 2007). These species play a crucial role in the development of different forms of damage in various human diseases (cancer, aging, neurodegenerative disease, malaria and pathological events in living organisms) (Gutteridge, 1994; Villamor et al., 2004).
The objective of this research was to validate the use of *S. crotalarioides* and *P. roseus* in traditional medicine; therefore, the anti-inflammatory and antioxidant properties and the acute toxicity of extracts of these plants in mice and rats were evaluated using various experimental models. This is the first attempt to observe whether both plant extracts have biological activity.

MATERIALS Y METHODS

Solvent and Reagents

Chloroform, ethanol and water were reactive grade. The reagents were Sigma-Aldrich. Indomethacin 99% TLC, carrageenan lamda type IV, Phorbol 12-myristate 13-acetate 99% TLC (TPA), Freunds adjuvant complete.

Plant material

S. crotalarioides was collected in the Las Comadres Municipality of Guadalcazar, San Luis Potosi State, Mexico in August 2007, and *P. roseus* was collected in El Chico, Hidalgo State, Mexico in September 2006. The taxonomic identification of the plants was confirmed by taxonomist José García Pérez. Voucher specimens (SPLM43012 and SPLM43013 respectively) have been deposited in the Isidro Palacios Herbarium of the Universidad Autónoma de San Luis Potosí.

The extracts were obtained by successive and exhaustive maceration to temperature of ebullition with chloroform, ethanol and water.

Animals

Male Wistar Rats (150-200 g) and male mice of the CD1 strain (20-25 g) from the Universidad Autónoma Metropolitana-Xochimilco animal facility were housed in isolated cages at 24 °C under a light-dark cycle of 12 h:12 h, and were maintained with food (Purina) and water *ad libitum*. The animals were performed according to the current procedure for the care of animals by the official Mexican Norm (NOM-062-ZOO-1999).

Anti-inflammatory evaluation

a) 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema

Edema was induced according to the method described by Young and De Young, 1989. A solution of 2.5 μg of 12-O-tetradecanoylphorbol 13-acetate (TPA) in 25 μL of acetone was topically applied to groups of male mice of the CD1 strain (20-22 g) on both the inner and outer surfaces of the right (W) and left ears (W'). Administration of *S. crotalarioides* and *P. roseus* extracts (2 mg/ear) and indomethacin (2 mg/ear) dissolved in acetone was performed 30 minutes after TPA administration to the right ear. The left ear was treated with the vehicle (acetone) only. The extent of inflammation in the control group was determined as the difference in the weight of the TPA-inflamed ear and the vehicle-treated ear (Wo). In all groups, the edema was allowed to develop for 6 hours. Then, the animals were sacrificed by cervical dislocation, and plugs (diameter of 6 mm) of the central portion were taken from both ears and weighed. The reduction of the edema in the mice treated with indomethacin or the extract was expressed using the equation:

$$\%\ inhibition = \frac{(W - Wo)}{(W' - W'o)} \times 100$$

b) Carrageenan-induced rat paw edema

The carrageenan-induced edema assay was carried out according to Winter et al., 1962. Groups of five rats were injected with 100 μL of a 1 % carrageenan solution in the sub-plantar region of the left hind paw. A test group of rats was treated orally with 50, 100,

200 or 400 mg/kg of one of the extracts 1 h prior to carrageenan injection.

At the same time, the control group received the vehicle (1 % Tween 80), while the standard reference group was treated with a solution of indomethacin (8 mg/kg). The paw volume was measured by the volume displacement method using a plethysmometer (Ugo Basile, Milan) at 1, 2, 3, 4 and 5 h after carrageenan administration. The inhibitory activity was calculated according to the following formula (Olajide et al., 2000):

$$\frac{(C_t - C_0)\quad control - (C_t - C_0)\quad treated}{(C_t - C_0)\quad control} \times 100$$

c) Adjuvant-induced arthritis in rats

The arthritic syndrome was induced in groups of seven rats (150-200 g) according to the method of adjuvant-carrageenan-induced inflammation (ACII) (Rocha et al., 2002) by injections of 0.1 mL of Freund's complete adjuvant intradermally at the base of the tail. The experimental group received chloroform extracts of *P. roseus* (100 mg/kg) or *S. crotalarioides* (50 mg/kg) in 1.0 % Tween 80, and the reference group received indomethacin (4 mg/kg) in the same delivery system. The control group received only vehicle. The animals were administered with indomethacin, extracts or vehicle daily in the morning for 6 to 10 days after adjuvant inoculation. On the sixth day, after one hour of drug administration, a suspension of 0.1 mL of carrageenan was injected in the subplantar region of the left hind paw of each rat. The paw volume was measured by the volume displacement method using a plethysmometer. These measurements were made before the adjuvant injection and were repeated again 6 days later at 3 and 5 h (acute phase) and were continued for 24 to 96 h after the carrageenan injection. The edema volume is expressed as the difference found in the left hind paw compared with the right hind paw. The inhibition of edema was calculated for each animal group in comparison with the control group.

d) Mouse ear edema induced by multiple topical applications of TPA

A solution of 2.5 μg of 12-O-tetradecanoylphorbol 13-acetate (TPA) in 25 μL of acetone was topically applied to groups of male mice of the CD1 strain (20-22 g) on both the inner and outer surfaces of the right (W) and left ear (W') 5 times over 10 days (Gábor, 2000). Topical administration of the extract (2 mg/ear) or indomethacin (0.5 mg/ear) dissolved in acetone on the left ear was performed 30 minutes after TPA administration. The extent of inflammation in the control group was determined as the difference of the weight between the TPA-inflamed ear and the vehicle-treated ear (Wo). All animals were sacrificed by cervical dislocation after 10 days, and plugs (diameter of 6 mm) of the central portion were taken from both ears and weighed. The reduction of edema in the mice treated with indomethacin or the extract was expressed using the equation:

$$\%\ inhibition = \frac{(W - Wo)}{(W' - W'o)} \times 100$$

Antioxidant potential

a) Free Radical Scavenging by the use of DPPH Radical.

The DPPH radical scavenging capacity of each extract was determined according to the method of Brand-Williams et al., 1995, as modified by Miliauskas et al., 2004. DPPH radicals have an absorption maximum at 517 nm, which disappears upon reduction by an antioxidant compound. The DPPH radical solution in methanol (9×10^{-5} M) was freshly prepared, and 2.9 mL of this solution was mixed with 100 μL of solutions of plant extracts at concentrations of 33, 16.5 and 8.25 μg/mL. The samples were incubated for 30 min at 37 °C in a water bath, and the decrease in absorbance at 517 nm was measured (A_E). A blank sample containing 100 μL of methanol in the DPPH radical solution was prepared daily, and its absorbance was measured (A_B). The radical scavenging activity was calculated using the following formula:

$$\%\ \text{inhibition} = 1 - [(A_B - A_E)/A_B] \times 100$$

b) Determination of Total Phenolic Content

The total phenolic concentration was determined using the Folin-Ciocalteu reagent according to Spanos and Wrosltad [12]. To 50 μL of each sample (three replicates), 2.5 mL of a 1/10 dilution of Folin-Ciocalteu´s reagent and 2 mL of Na_2CO_3 (7.5 %, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-Vis spectrophotometer. The results are expressed as milligrams of gallic acid equivalent per gram of dry weight extract (mg GAE/g dw).

c) Determination of Reducing Power

The reducing power was determined according to the method described by Oyaizu, 1986. A 0.125 mL aliquot of various concentrations of extract (33, 16.5 and 8.25 µg/mL) was mixed with 1.25 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1.25 mL of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After 1.25 mL of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650*g* for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride, and the absorbance at 700 nm was measured. The obtained observance was compared with the ascorbic acid value as a standard.

Acute toxicity

The extract was orally administered as a single dose to groups of mice (n = 3) at different concentrations (1000-5000 mg/kg). The range of doses used in mice followed the method of Lorke, 1983. After administration, the animals were observed under open-field conditions for a 72 h period. The number of animal deaths and signs of clinical toxicity were recorded during the first 24 h. The median lethal dose (LD_{50}) and 95 % confidence limits were calculated by the method of Litchfield and Wilcoxon, 1949.

Statistical analysis

Statistical analysis was performed using the Student's t-test ($P < 0.05$), while ANOVA followed by Dunett's multiple comparison test was used in order to compare more than two groups.

RESULTS AND DISCUSSION

The yields of the chloroform, methanol and aqueous extracts of *Senna crotalarioides* and *Penstemon roseus* are shown in Table 1. The chloroform extracts provided the highest yield.

Table 1. Yield of the extracts of *S. crotalarioides* and *P. roseus*

Extracts	Yield (%)
Senna crotalarioides	
Chloroform	2.20
Methanol	1.24
Aqueous	2.07
Penstemon roseus	
Chloroform	5.06
Methanol	4.90
Aqueous	3.95

Table 2 shows the inhibitory effects of the chloroform, methanol and aqueous extracts of *S. crotalarioides* and *P. roseous* on TPA-induced ear edema. It is evident that the aqueous extracts of both plants at doses of 2 mg/ear did not produce a significant effect; however, the methanol and chloroform extracts of both plants had significant activity, and we decided to follow-up with both chloroform extracts, so the anti-inflammatory effect was tested in models of acute and chronic inflammation.

Table 2. Effects of the topical application of indomethacin and chloroform, methanol and aqueous extracts of *S. crotalarioides* and *P. roseus* on TPA-induced ear model in mice.

Treatment	Dose (mg/ear)	Edema formation (mg)	Inhibition (%)
Control	0	12.27±0.35	0
S. crotalarioides			
Chloroform	2	7.82±0.48*	36.27±3.93
Methanol	2	8.26±0.44*	32.68±3.61
Aqueous	2	8.70±0.43	29.09±0.87
P. roseus			
Chloroform	2	6.95±0.36*	43.36±1.23
Methanol	2	7.77±0.50*	36.67±3.71
Aqueous	2	8.91±0.64	27.38±2.99
Indomethacine	2	6.28±0.56*	48.82±5.70

Results are mean ± S.E.M., * extract and the reference, $p< 0.05$ vs control according T Student's test.

The results obtained with the chloroform extracts of *S. crotalarioides* (CSC) and *P. roseus* (CPR) on mouse ear edema induced by multiple topical applications of TPA at a dose of 2 mg/ear (Table 3) demonstrated that both extracts exhibited anti-inflammatory effects. The activity of the chloroform extracts of *S. crotalarioides* (64.92 %) was similar to that of indomethacin at a dose of 0.5 mg/ear (62.74 %), and CPR showed a minor effect (42.30 %).

Table 3. Effect of the chloroform extracts of *S. crotalarioides* and *P. roseus* on chronic inflammation induced by TPA in mouse ear.

Treatment	Dose (mg/ear)	Edema formation (mg)	Inhibition (%)
Control	0	10.52±0.77	0
S. crotalarioides	2	3.69±0.27*	64.92
P. roseus	2	6.07±0.51*	42.30
Indomethacin	0.5	3.92±0.44*	62.74

Results are mean ± S.E.M., * extracts and the reference $p< 0.05$ vs control according T Student's test.

Table 4. Effect of chloroform extracts of *S. crotalarioides* and *P. roseus* on paw edema of rats induced with carrageenan.

Treatment	Dose mg/kg	Paw oedema (% Inhibition)				
		1h	2h	3h	4h	5h
Control		(0)	(0)	(0)	(0)	(0)
S. crotalarioides	400	N.E	N.E	N.E.	N.E.	N.E.
	200	N.E.	N.E.	N.E.	N.E.	N.E.
	100	N.E.	N.E.	N.E.	N.E.	N.E.
	50	55.78±12.07*	62.82±17.26*	50.92±12.76*	41.67±15.89	28.03±10.84
P. roseus	400	N.E.	31.41±4.26	37.61±4.26	28.80±10.90	N.E.
	200	20.83±5.27	29.30±4.02	52.31±4.47*	40.00±3.75	41.20±3.42
	100	38.31±8.53	43.69±9.38*	36.92±7.89	34.80±4.33	34.09±3.51
	50	47.37±11.67	26.92±6.08	40.17±14.74	35.66±6.64	N.E.
Indomethacin	8	N.E.	26.92±7.28	57.40±4.58*	56.00±4.03*	56.29±5.22*

Results indicate percent reduction in paw volume compared with the control group treat only carrageenan±standard error. n= 7. Dunnett test * p< 0.05. N.E= not effect.

The pronounced inflammation induced by TPA when administered topically is thought to be mediated by protein kinase C and the stimulation of phospholipase A_2 (Füstenberger et al., 1981), which results in the release of arachidonic acid and prostaglandin E_2 (Ashendel and Boutwell, 1982). Thus, both phospholipase A_2 and cyclooxygenase inhibitors are effective in these models, and CSC and CPR may interfere with these mediators to inhibit TPA-induced inflammation.

Both extracts, tested *in vivo*, showed significant activity on carrageenan-induced inflammation with respect to the control group (Table 4). CSC showed no activity at doses of 100, 200 and 400 mg/kg; however, a dose of 50 mg/kg produced a significant reduction of 55.8 % after 1.0 h, and the effect was unchanged for 3 h, decreased after 4.0 h (36.0 %), and was eliminated after 5 h. CPR showed the best effect at a dose of 200 mg/kg 3 h after the administration (52.3 %).

It is well known that leukocyte migration to injured tissue is an important aspect of the inflammatory process. The release of several mediators of the flogistic response, such as histamine and serotonin, are responsible for the immediate inflammation response (Crunkhon and Meacock, 1971), whereas kinins and prostaglandins mediate the prolonged response (Brito and Antonio, 1998). On the other hand, some plant constituents can significantly inhibit the biosynthetic pathways of inflammation mediators (Speroni et al., 2005). Currently, the hypothesis is that both extracts can significantly inhibit the production of these mediators.

Rheumatoid arthritis is a chronic progressive autoimmune inflammatory disease of unknown etiology and complex multifactorial pathogenesis characterized by irreversible erosive synovitis, resulting in deformity, loss of joint space and function, and bone destruction (Rassol amd Varalakshmi, 2007). Increased edema in rat hind paw injected with carrageenan has an acute and a prolonged phase and has been proposed as a suitable and simple model system for evaluating the anti-inflammatory activity and therapeutic effects of treatment (Billiau and Matthys, 2001; Lee et al., 2004). The above results showed that CSC had no activity on adjuvant-induced arthritis in rats (Table 5). However, CPR exhibited an anti-arthritic effect in the acute phase. After 24 h of oral administration of the extract at doses of 100 mg/kg, the inhibition was 69.17 %, which was similar to that obtained in animals treated with indomethacin (4 mg/kg), but the effect decreased after 48 h (32.45 %) and had no effect after 72 h.

Table 5. Effect of the chloroform extracts of *S. crotalarioides* and *P. roseus* on rat treatment with Freud adjuvant and paw edema induced with carrageenan.

Time (h)	Treatment and % Inhibition			
	Control	*S.crotalarioides 50 mg/kg*	*P. roseus 100 mg/kg*	Indomethacin 4 mg/kg
3	0	N.E.	N.E.	34.10±6.43
5	0	N.E.	N.E.	45.95±9.62*
24	0	N.E.	69.17±2.14*	75.53±4.59*
48	0	N.E.	32.45±7.56	48.10±3.46*
72	0	N.E.	N.E.	55.03±8.77*
96	0	N.E	N.E.	36.89±9.25

Results indicate percent reduction in paw volume compared with the control group treat only carrageenan. ± standard error of increase in paw volume. n= 7. Dunnett test * p< 0.05. N.E= not effect.

The antioxidant capacity of the extracts of *Senna crotalarioides* and *Penstemon roseus* was determined by the radical scavenging based on the DPPH assay. The methanol extracts of *S. crotalarioides* and *P. roseus* were most active, with inhibitory concentrations (IC_{50}) of 23.04±0.22 and 19.27±0.37 µg/mL, respectively; these values were higher than that of ascorbic acid, at 0.58±0.02 µg/mL. The total phenol content was evaluated and the aqueous extract of *S. crotalarioides* showed the major concentration of these kinds of compounds (253.37±7.65 µg/mL). This amount represents twice the values obtained with every extract processed. The reducing power of methanol extracts showed the major activity with respect to ascorbic acid as a reference (13.99±4.37 and 14.98±0.69 % for *S. crotalarioides* and *P. roseus*, respectively) (Table 6). The antioxidant capacity of different medicinal plants and herbs has been linked to *in vivo* protection against oxidative stress in numerous studies (Prior et al., 2005), but has rarely been associated with an anti-inflammatory capacity (Jensen et al., 2008).

Table 6. Antioxidant capacity of the extracts of *S. crotalarioides* and *P. roseus*

Treatment	DPPH	Total phenols	Reducing power (%)
S.crotalarioides			
Chloroform	88.62±0.86	69.87±1.35	5.84±0.12
Methanol	23.04±0.22	137.52±4.10	13.99±4.37
Aqueous	43.29±1.16	253.37±7.65	6.53±0.13
P. roseus			
Chloroform^{o}	270.32±36.94	115.67±8.75	1.87±0.23
Methanol	19.27±0.37	126.82±6.00	14.98±0.69
Aqueous	171.53±10.42	120.12±10.00	5.61±0.66
Quercetin	-	782.92±36.80	7.63±1.11
Ascorbic acid	0.58±0.02	-	100

Data are expressed as mean ± error standar (n=3). DPPH radical solution in methanol (9 x 10^{-5} M, 35.48 µg/mL) using (33µg/mL) of plant extract and ascorbic acid (5 µg/mL). Total phenols expressed in mgs. of Gallic acid equivalent per g of sample (mgGAE/g). Reducing power is expressed with respect to ascorbic acid (33µg/mL).

In Table 7, the LD_{50} of the chloroform extracts of CSC (2150 mg/kg) and CPR (3,800 mg/kg) in mice are shown. The values obtained are considerate as slight acute toxic and both doses are higher than those used in the different models in this study.

Table 7. Evaluation of LD_{50} of the chloroform extracts of *S. crotalarioides* and *P. roseus* in mice.

Chloroform extracts	LD_{50} (mg/kg)
Senna crotalarioides	2,150
Penstemon roseus	3,800

CONCLUSIONS

Senna crotalarioides and *Penstemon roseus* or their constituents could represent, in the near future, a new therapeutic option for the treatment of inflammatory diseases, and in the case of *P. roseus*, might be important in the treatment of chronic progressive autoimmune inflammatory diseases. Both plants also have antioxidant capacity, and the activities were comparable to those of known antioxidant ascorbic acid, which has been linked to protection against oxidative stress.

S. crotalarioides and *P. roseus* did not present acute toxicity at the studied doses. Experiments are in progress to identify the active compounds and to further evaluate the molecular mechanism through which these compounds exert their anti-inflammatory and antioxidant properties.

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