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Antioxidant Activity In Rhizomes From *Smilax Campestris* Griseb. Smilacaceae

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ABSTRACT

The rhizomes from several species of *Smilax* contain several compounds, which have been reported to show antioxidant activity. Among these compounds, proanthocyanidins may be one of the secondary plant metabolites to which the antioxidant activity can be related to.
The aim of this work was to establish if the rhizomes of *Smilax campestris* Grsieb. growing in Argentina show antioxidant activity and the variations of this activity throughout the year.
Plant material in different phenologic states came from Puerto Gaboto (Santa Fe Province, Argentina). Total reactive antioxidant potential (TRAP) was measured by chemiluminescence, using 2,2- azobis 2-amidinopropane (ABAP) as the source of free radicals.
The results showed that the antioxidant capacity in the extracts of the rhizomes followed a circannual pattern. The highest antioxidant activity was measured in February to decreased significantly in the following months. During pre-flowering the activity increased again, with a slight decrease after flowering and again increased during fruiting.

Key Words: *Smilax campestris*, "zarzaparrilla blanca", proanthocyanidin, antioxidant capacity, phenologic states.

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INTRODUCTION

The genus *Smilax* belongs to the Smilacaceae, a family of subwoody rhizomatous dioecious twining plants, with about 300 species growing in the tropical hot regions of both hemispheres. A great number of them grow in Southeastern Asia and South America. In South America it occurs in Brazil (southern forests), Bolivia, Paraguay, Uruguay and Argentina.

In Argentina eight species are present: *S. campestris* Griseb., *S. rubiginosa* Griseb., *S. campestris* var. *rubiginosa* (Griseb) A. D.C., *S. asumptionis* A. D.C., *S. cognata* Kunth, *S. brasiliensis* Sprengel, *S. fluminensis* Stendel y *S. pilcomayensis* Guaglian. et Gattuso. Their distribution area includes northern provinces, as the forests in Misiones and the tucumano - oranense forest. Towards the East, they reach the Rio de la Plata coast (Guaglianone and Gattuso, 1991).

During the XIX century, different *Smilax* species have been included in the Pharmacopoeias from several countries, such as the USP and the British Pharmacopoeia. The roots and rhizomes from *S. medica* Cham. et Schlecht, *S. ornata* Hook, *S. papyraceae* Duhamel y *S. officinalis* Kunth (Liliaceae) were incorporated as official drugs under the names "zarzaparrilla" or "sarsaparrilla" in the 2nd edition of the National Argentine Pharmacopoeia.

S. campestris Griseb. is ordinary known as "zarzaparrilla blanca". Its roots and rhizomes were used in folk medicine for syphilis treatment, in rheumatism and some skin deseases; further as diaphoretic and diuretic. The leaves and aerial stems were used to elaborate tonic, bitter, digestive drinks (Mandrile and Bongiorno de Pfirter, 1991).

In rhizomes from some *Smilax* species growing in Central America, *S. lundellii* Killip et C.V. Mortony and *S. spinosa* Miller, the presence of compounds with suggested antioxidant activity, such as leucoanthocyanidin and proanthocianidin, has been reported (Cáceres, 1998). Previous work performed in our laboratories showed that *S. campestris* rhizomes during the secondary plant metabolism proanthocianidins such as procyanidin and propelargonidin are produced (Rugna, *et al.*, 2002a).

The aim of this work was to establish if the rhizomes of *Smilax campestris* Grsieb. growing in Argentina show antioxidant activity and the variations of this activity throughout the year.

EXPERIMENTAL

Plant material

Plant material came from Puerto Gaboto (Santa Fe Province, Argentina), collected in several months and in different phenologic states. Voucher specimens are kept in the Herbarium belonging to the Museo de Farmacobotánica "Juan A. Domínguez", Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (BAF N° 4565).

Preparation of extracts

2 g of air-dried pulverized rhizomes were extracted at room temperature with methanol 50% (Watterman and Mole, 1994); the extracts were concentrated to dryness under reduced pressure by means of a rotatory evaporator and then analyzed employing standard methods for isolation and characterization of flavonoids (Mabry *et al*., 1970; Markham, 1982; Watterman and Mole, 1994).

Antioxidant Capacity Determination

Total reactive antioxidant potential (TRAP) was measured by chemiluminescence as described by Lissi et al. in 1992. Briefly, the reaction medium used contained 20 mM 2,2- azobis 2- amidinopropane (ABAP) and 40 μM luminol. ABAP is a source of free radicals that react with luminol yielding chemiluminescence which was measured in a Packard Tri Carb liquid scintillation counter. The addition of a sample aliquot decreases the chemiluminescence for a period proportional to the amount of antioxidants present in the sample, to basal levels until luminol radicals are regenerated (induction time, δ). The system was calibrated against catequin,(Fig 1) and the results were expressed in μM catequin.

RESULTS AND DISCUSSION

As it is shown in Figure 2, the antioxidant capacity of the rhizomes from *S. campestris* is changed throughout the year. The highest antioxidant activity was measured in February to decreased significantly in the following months. During pre-flowering the activity increased again, with a slight decrease after flowering and again increased during fruiting. These variations in the concentrations of antioxidant compounds may be related to the phenologic state and the time of the year.

Previous research performed on whole *S. campestris* plants showed that no matter the sex the flavonoid production changed along the year. The main values were obtained during flowering, which indicates that the increase in the flavonoid production may be associated with an increase of metabolic activity (Rugna *et al.*, 2002b).

According to the results obtained by Rugna *et al.* (2002a), during flowering and fruiting the production of proanthocyanidins is the highest of all the periods studied, and the variation of antioxidant capacity showed the same pattern. These results suggest that when intending to use extracts of rhizomes from *S. campestris* as antioxidant agents, it is very important to take into account the collection time because this fact is related

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with the circannual metabolism of the plant, in other words, its phenologic state.

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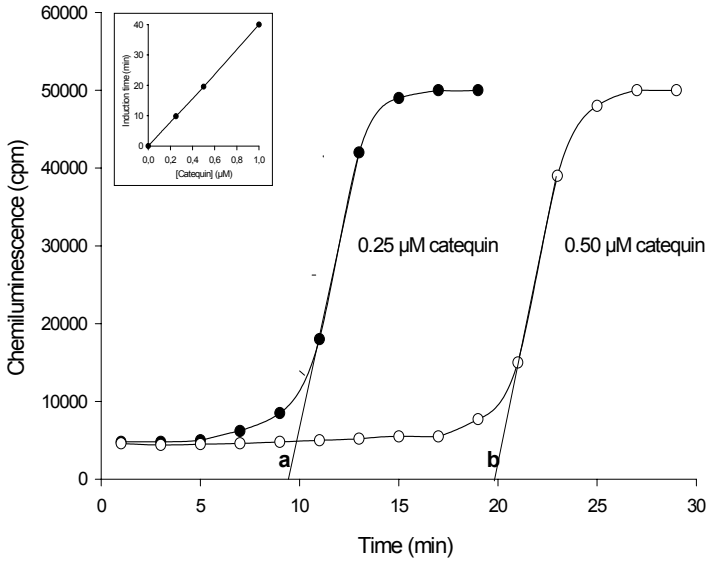

Figure 1: Time profiles of luminol chemiluminescence of the ABAP-luminol system from two different catequin concentrations. Inset: Dependence of induction time on Catequin concentration .

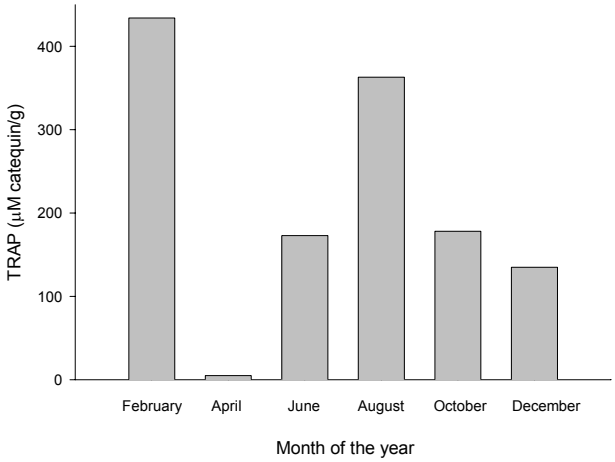

Figure 2: Changes in the total antioxidant capacity of the extracts of rhizomes de *S. campestris* throughout the year.

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CONCLUSIONS

1. The antioxidant capacity ranged between 5 and 440 μM catequin/ g of extract, according to the time of the year when the sample was collected.

2. In rhizomes, the antioxidant activity increases during flowering and fruiting, with values from 200 μM catequin / g of extract to 440 μM catequin / g of extract.

3. In order to use extracts of rhizomes from *S. campestris* as antioxidant agents, it is important to take into account the collection time because this fact is related with the circannual metabolism of the plant, in other words, its phenologic state.

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