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Purifying capacity of free radicals and cytotoxicity of plant extracts

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INTRODUCTION

Free radicals are chemical species that show an odd electron in the external orbital of the atomic structure, giving it a space configuration that gives rise great unstability.

The oxidative damage or stress occurs when the living matter is exposed to diverse sources that either produce a balance rupture that should exist among substances or prooxidant factors, and the antioxidant mechanisms in charge of eliminating these chemical species, because of a defense deficit or a high increment of reactive oxygen species production. Consequently, structure-function relationship in any organ, system or specialized cellular group is altered. Therefore, it is recognized as a general mechanism of cell damage, associated to physiopathologic processes (Aruoma, 1996).

Phenolic compounds (PC) from plants are an inexhaustible source of natural antioxidants. Natural polyphenols include simple molecules (phenolic acids, phenylpropanoids, flavonoids) and polymeric compounds (lignins, tannins), all can delocalize electrons in their molecules and exhibit a wide range of biological effects: antibacterial, anti-inflammatory, hypolipidemiant, antialergic, anticarcinogenic, etc; many of these biological effects were attributed to their free radical capture capacity.

The aim of this work is to determine the antioxidant capacity of acqueous and alcoholic extracts of *Caesalpinia paraguariensis*, *Verbascum virgatum* and *Cestrum parqui*, native plant species popularly used for the treatment of ailments and affections, and to estimate the degree of cytotoxicity of their extracts to support their medicinal use.

The test was carried out in triplicate.

The reaction mixture was protected from light, for 20 min, $OD_{lambda514}$ was read (Beckman DU650). The calculations of % neutralization (W) were performed according to:

$W = (A_c\text{-}A_M) \times 100/A_c$; where, A_c is $OD_{lambda514}$ of the reaction mixture without M(n), A_M is 1 $OD_{lambda514}$ of the reaction mixture plus M(n).

Scavenging rate of DPPH. The most active extracts (*C. paraguariensis*) were selected for this test, the concentrations of extracts were standardized. The $OD_{lambda514}$ was measured at intervals of 15 sec for 10 min.

Cytotoxicity. By the lethality test of saline *Artemia*, the effect of the extracts was proved with the best activity AO on living larvae. Data (survivor number after 17 h exposition) were processed to obtain the lethal 50% dose (LD_{50}) (Mc Laughlin, 1991).

MATERIALS AND METHODS

Plant material was collected in time and appropriate form for each species. It was dried off, and powdered to prepare extracts, infusion (I), decoction (D) and tinctures (T), according to the techniques of the Pharmacopeia Argentina 6th Ed. They were kept at -20°C. The PC content of the extracts was determined by Folin-Ciocalteu reagent, using coumarin like standard. Quercetin and BHT (Sigma®) were used like positive controls.

Method of 1,1-diphenyl -2-picrylhydrazyl (DPPH).

The DPPH molecule is characterized as a stable free radical because of being able to delocate its odd electron. Its violet colour is due to this delocalization, whose absorption band, in ethanol, is centered at a *lambda* = 514-520 nm. When the DPPH solution is mixed with a substance able to donate a hydrogen atom, the molecule is reduced to the diphenylpicrylhydrazine form, losing the violet colour (Hu Fenglin *et al.*, 2004).

DPPH was used in ethanol 96° (0.03 mg/ml), concentrations of extracts were tested between 0.5 and 10 ppm PC. The reagents were added in the following order:

Table 1. Reaction protocol

Tubes	Samples	Ethanol 96	DPPH
Blanck	-	2 ml	-
Control	-	1.5 ml	0.5 ml
M1	0.1 ml	l.4 ml	0.5 ml
M2	0.1 ml	l.4 ml	0.5 ml

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RESULTS AND DISCUSSION

EC_{50} and EC_{90} were compared in Table 2 regarding positive controls of antioxidant activity, quercetin (natural) and BHT (synthetic).

Table 2. Purifying capacity of DPPH

Especies(NC)	UP	EF	EC_{50}	EC_{90}
C. *paraguariensis*	Bark	Infusion	1.0	4.5
		Decoctio	1.5	3.5
		Tinctur	1.0	4.5
V. virgatum	Leaves	Infusion	5.0	NR
		Decoctio	3.5	NR
		Tinctur	>10 (ND)	NR
C. *parqui*	Leaves	Infusion	3.1	NR
	Flowers	Infusion	3.0	NR
Quercetin	-	-	0.9	4.0
BHT	-	-	60.0	NR

References: UP: used part; EF: extractive form; Phenolic compounds (ppm); ND: not determined; NR: not reached.

EC_{50} is the concentration that is able to neutralize the 50% of DPPH, a useful parameter to compare the mean activity of the extracts. EC_{90} is the concentration able to neutralize 90% of DPPH radicals, revealing which are the substances able to reach 90% of purifying activity, at concentrations ≤ 10 ppm, is most representative of the antioxidant power of a given sample than EC_{50}. Then, the extracts of *C. paraguariensis* (I: 1.0; D: 1.5 and T: 1.0 ppm PC*), have radical capture activity comparable to quercetin (0.9 ppm), the extracts of *V. virgatum* and *C. parqui* demonstrated good purifying capacity (IV: 5, CV: 3.5, IFP: 3.0 and IHP: 3.1 ppm) Tincture of *V. verbascum* didn't reach the EC_{50} with 10 ppm, and all overcame BHT (60 ppm). Only reached EC_{90} the extracts of *C. paraguariensis* (I: 4.5; D: 3.5 and T: 4.5 ppm) and quercetin (4.0 ppm).

The respective DPPH capture rates are shown in Fig. 1.

The % inhibition at 15 sec were: IG: 52.1%, CG: 39.0%, TG: 24.6%, quercetin: 29.2%. IG reaches 50% of its activity in 15 sec, CG in 30 sec overcoming the antioxidant control (quercetin 1 min) and TG (1.5 min).

The EC_{90} was reached by IG in 4 min, CG in 10 min, quercetin in 6 min; TG didn't reach EC_{90} in the 10 minutes of measurement.

Cytotoxicity. The LD_{50} values obtained for the acqueous extracts were two orders higher than EC90. TG was cytotoxic.

Table 3. CYTOTOXICITY

	LD_{50}
Infusion *C. paraguariensis*	2,570.6
Decoction *C. paraguariensis*	1,880.0
Tincture *C. paraguariensis*	0.0

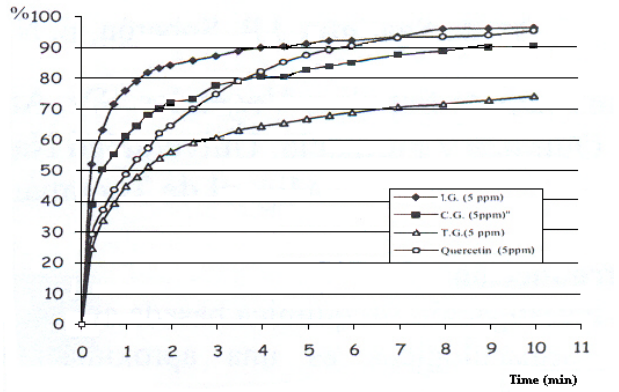

Figure 1. Scavenging rate of DPPH

CONCLUSIONS

Of the tested extracts, Infusion of *C.paraguariensis* demonstrated to have the best capture capacity of free radicals to act with the lowest concentration in the minor time. It is also the less cytotoxic of the tested extracts.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina,
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