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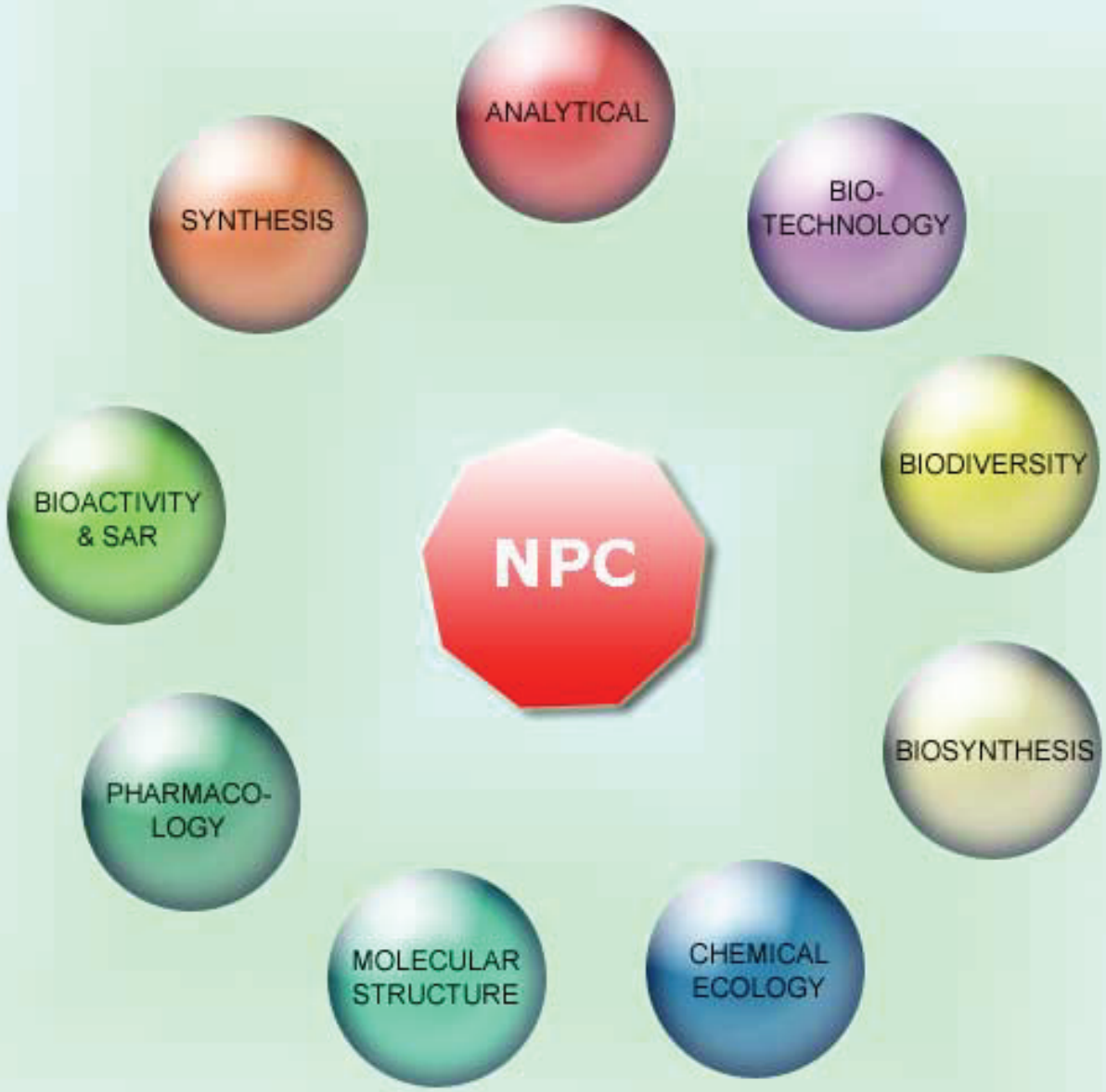

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Antioxidant, Hemolytic and Cytotoxic Activities of *Senecio* Species used in Traditional Medicine of Northwestern Argentina

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Senecio nutans Sch. Bip., *S. viridis* var. *viridis* Phill. and *S. spegazzinii* Cabrera are native species used in traditional medicine of northwestern Argentina. The total phenolics, flavonoids and caffeoylquinic acids contents, as well as radical scavenging, antioxidant, hemolytic and cytotoxic activities of aqueous extracts (infusion and decoction) of all three species were determined. *S. nutans* was the most active. The extracts did not show antibacterial activity. Alkaloids were not detected in any of the aqueous extracts of the three studied species.

Keywords: *Senecio nutans*, *Senecio viridis*, *Senecio spegazzinii*, Total phenolics, Caffeoylquinic acids, Antioxidant, Radical scavenging, Cytotoxic activity.

Numerous plant species have been demonstrated to exhibit powerful antioxidant properties. About 267 *Senecio* species (Asteraceae) have been reported for Argentina, and among them, 42 are toxic to cattle [1a] and only four are used in traditional medicine [1b]. In this work we analyzed the total phenolics, flavonoids and caffeoylquinic acids contents and also the radical scavenging, antioxidant, hemolytic and cytotoxic activities of aqueous extracts (infusion and decoction) of the three *Senecio* species most frequently employed in traditional medicine of northwestern Argentina. *S. nutans* Sch. Bip is commonly named “chachacoma”. Its leaves are used as a gastric antispasmodic and for the treatment of gastric ulcers. The infusion is claimed to be useful in the treatment of altitude sickness. It is an edible species used as a flavouring agent, and also employed as a veterinary antiparasitic [1b-d]. *S. viridis* var. *viridis* Phil., commonly known as "mocora", “mocoraca” or “chachacoma del burro”, is an aromatic shrub. The leaves are chewed to alleviate toothache [1b]. *S. spegazzinii* Cabrera, a herbaceous plant, commonly known as “salvia de la puna” or "chachacoma”, is very scarce and used to treat earache and colds [1b].

Infusions and decoctions from *S. nutans* showed the highest contents of phenolic compounds (20.5 and 25.2 mg GAE/g dry leaf), flavonoids (8.7 and 7.1 mg QE/g dl) and caffeoylquinic acids (15.9, and 18.6 mg CAE/g dl), respectively. The phenolic contents of *S. viridis* and *S. spegazzinii* were approximately a half of those found for *S. nutans* (Table 1). Four methods have been employed to measure the antioxidant activity: DPPH radical scavenging, ABTS radical scavenging, β-carotene-linoleate bleaching assay and total antioxidant capacity [Mo(VI) to Mo(V) reduction test]. Again, the aqueous extracts of *S. nutans* showed to be the most active in the four methods assayed. The results are summarized in the Table 2 and 3. The infusions and decoctions from the three *Senecio* species assayed showed no hemolytic activity against human and rabbit erythrocytes. Both infusion and decoction of *S. nutans* exhibited low toxicity against *Artemia salina* nauplii. The test showed that lethality (LC_{50}) of brine shrimp larvae was 451.3 µg/mL and 437.1 µg/mL for infusion and decoction respectively.

Table 1: Total phenolics, flavonoids and caffeoylquinic acid contents in *Senecio nutans, S. viridis* and *S. spegazzinii*.

Extracts	Weight* (mg)	Total phenolics (mg GAE/g dl#)	Total flavonoids (mg QE/g dl#)	Caffeoylquinic acids (mg CAE/g dl#)
S. nutans (Inf)	452	20.5 ± 0.2	8.7 ± 0.3	15.9 ± 0.2
S. nutans (Dec)	512	25.2 ± 0.4	7.1 ± 0.1	18.6 ± 0.6
S. viridis (Inf)	513	9.6 ± 0.2	2.5 ± 0.2	8.3 ± 0.2
S. viridis (Dec)	525	11.2 ± 0.8	3.4 ± 0.1	9.4 ± 0.3
S. spegazzinii (Inf)	525	8.6 ± 0.1	-	6.5 ± 0.1
S. spegazzinii (Dec)	513	10.7 ± 0.1	-	8.7 ± 0.9

* Weight of lyophilized residue obtained from 2.0 g of dry leaf (dl) after aqueous extraction; Inf: Infusion; Dec: Decoction; #dl: dry leaf.

Table 2: Antiradical activity of aqueous extracts from *Senecio nutans*, *S. viridis* and *S. spegazzinii*.

Extracts	DPPH• scavenging IC_{50}value (µg/mL)#	ABTS•+ scavenging IC_{50}value (µg/mL)#
S. nutans (Inf)	22.2	11.8
S. nutans (Dec)	32.6	10.2
S. viridis (Inf)	36.0	20.6
S. viridis Dec	34.2	20.8
S. spegazzinii (Inf)	29.5	40.5
S. spegazzinii (Dec)	24.1	42.8
BHT	20.1	3.9

#µg of lyophilized residue obtained from infusion or decoction.

Table 3: Antioxidant activity of aqueous extracts from *Senecio nutans, S. viridis* and *S. spegazzinii*.

Extracts	β-carotene/linoleic acid inhibition at 47 µg/mL#	Total antioxidant capacity Mo(VI) to Mo(V) test	
		µg AAE*	µg QE**
S. nutans Inf	67%	28	54
S. nutans Dec	84%	36	71
S. viridis Inf	62%	35	70
S. viridis Dec	83%	46	94
S. spegazzinii Inf	60%	39	77
S. spegazzinii Dec	80%	46	93
BHT	94%	-	-

#These µg refer to micrograms of lyophilized residue obtained from infusion or decoction; *AAE: ascorbic acid equivalents; **QE: quercetin equivalents per gram of lyophilized residue.

On the other hand, the decoction of *S. viridis* var. *viridis* exhibited very low toxicity (LC_{50} 833.6 µg/mL), while the infusion showed

Table 4: Toxicity activity against *Artemia salina*.

Extracts	LC_{50} (µg/mL)*		
	S. nutans	*S. viridis*	*S. spegazzinii*
Infusion	451.3	>1000	>1000
Decoction	437.1	833.6	>1000
$K_2Cr_2O_7$		23.7	

*µg refers to micrograms of lyophilized residue obtained from infusion or decoction.

no toxicity (LC_{50} > 1000 µg/mL) against *A. salina*. The aqueous extracts of *S. spegazzinii* were non toxic (Table 4).

Alkaloids were not detected in any of the aqueous extracts from the three studied species (Dragendorff and Mayer reagents). The aqueous extracts did not show antibacterial activity against *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *Echerichia coli* ATCC 25922, *E. coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. As expected, the results obtained show that the antioxidant activity of the aqueous extracts from the *Senecio* species studied here is directly related to the amount of total phenolics, which are mostly constituted by caffeoylquinic acids (Table 1). The brine shrimp lethality assay results, the lack of hemolytic activity and the absence of alkaloids suggest that infusions and decoctions of these three *Senecio* species are essentially devoid of toxicity, in agreement with their ancient use in Andean traditional medicine for which there are no reports of unwanted acute events after their consumption.

Experimental

Preparation of the extracts: The aqueous extracts were prepared as infusions (Inf) and decoctions (Dec). Infusions were prepared by pouring 100 mL of boiling water onto 2.0 g of air-dried leaves. The mixture was left at room temperature for 20 min and filtered. Decoctions were prepared by refluxing 2.0 g of leaves with 100 mL of water for 20 min. After cooling to room temperature, the mixture was filtered. All extracts were lyophilized separately and stored at 4°C until use.

Determination of total phenolic, flavonoid and caffeoylquinic acid contents: The total phenolic, flavonoid and caffeoylquinic acid contents were determined using the methods of Folin–Ciocalteu [2a], Arvouet-Grand *et al.* [2b], and Martino *et al*. [3], respectively. Total phenolics, flavonoids and caffeoylquinic acids were expressed as mg gallic acid, quercetin and chlorogenic acid equivalents (mg GAE, mg QE and mg CAE respectively) per gram of dry leaf (g dl). The values are presented as means of triplicate analyses.

Antioxidant activity: The free radical scavenging activity was determined spectrophotometrically by the DPPH scavenging assay [4a]. The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re *et al.*, with some modifications [4b]. BHT was used as the antioxidant standard for comparison of the activity. Antioxidant activity of the extracts was determined according to a slightly modified version of the β-carotene-linoleate bleaching method [5a]. The percentage of inhibition was computed according to the formula proposed by Kulisic *et al*. [5a]. The total antioxidant capacity of the extracts was evaluated by the phosphomolybdate method [5b].

Hemolytic activity: The hemolytic activity was determined by the method proposed by Cantillo *et al.*, with some modifications [6]. Different concentrations of extracts were assayed against human and rabbit erythrocytes. The percentage of hemoglobin dissolved was determined spectrophotometrically. Triton X-100 was employed as a positive control.

Toxicity against *Artemia salina* Leach*:* The brine shrimp lethality assay was performed by the method of McLaughlin and coworkers [7]. Brine shrimp eggs (*Artemia salina*) were hatched in saline solution (3.8%). The extracts were tested at concentrations of 1000, 500, 100 and 10 µg/mL. Survival was measured after 24 h incubation at 10°C. Potassium dichromate was used as reference. The collected data were computerized and LC_{50} values determined by Probit analysis.

The level of toxicity of the extracts was defined according to the following categories: extremely toxic (LC_{50}<10 µg/mL), very toxic (10<LC_{50}<100 µg/mL), moderately toxic (100<LC_{50}<1000 µg/mL) and non toxic (LC_{50}>1000 µg/mL) [8].

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