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Nectandra megapotamica (Spreng.) Mez.: phytochemical characterization and neutralizing effect on Bothrops diporus venom

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Nectandra megapotamica (Spreng.) Mez.: phytochemical characterization and neutralizing effect on *Bothrops diporus* venom

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Antisnake activity of extracts and essential oil of *Nectandra megapotamica* was tested *in vitro* against the *Bothrops diporus* venom (*yarará chica*). Inhibitory activity of the hemolytic action on the aqueous extract and essential oil; inhibition of the procoagulant action on hexanic extract and condensed water from steam distillation; and inhibition of the proteolytic activity on alcoholic extract and condensed water was found. In all cases, the main antisnake venom activity was found on plant material collected in the autumn. The chemical composition of the *N. megapotamica* essential oil was characterized during different vegetative states finding a clear predominance of mono- (21.0–31.7) and sesquiterpene (58.5–68.9) hydrocarbons. The differences found with previous results published for this species, growing in other geographic places, opens the option for the existence of chemical types.

Keywords: *Nectandra megapotamica*; *Bothrops diporus*; antisnake venom activity; essential oil; chemical characterization

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Introduction

Nectandra megapotamica (Lauracea), commonly known as *canela preta*, *laurel hu*, *canela-fedorenta*, *laurel negro canelinha* and *canela-amarela*, is a South American species that is spreading from the southern parts of Brazil to the northern part of Argentina (Corrientes, Entre Ríos, Formosa, Misiones) and Paraguay and Uruguay (1).

The wood is used for civic and naval constructions (2, 3), while anti-inflammatory and analgesic activities were verified on an alcoholic leaf extract (4). Previous phytochemical studies on samples of these extracts have reported the isolation of α-asarone, galgravine and veraguensin, with analgesic and anti-inflammatory effects on mice (4); as well as tetrahydrofurane lignanes, which proved to possess *in vitro* antileishmanial, antimalarial and trypanocidal activities (5, 6).

Nectandra megapotamica essential oils have been reported due to their pharmacological properties as antitumoral, anti-inflammatory and antimicrobial (7). Romoff et al. (8) reported, for the first time, the chemical composition of the volatile oils from *N. megapotamica* leaves collected in São Paulo (São Paulo State, Brazil), in two distinct months at different hours of a day, identifying nineteen components with predominance of sesquiterpenes as δ-elemene (8.2–22.6%) and oxygenated sesquiterpenes as α-bisabolol (62.3–69.4%) being noticeable by the fact that the content on oxygenated compounds was higher in February (summer) (70%) compared with samples collected in August (autumn) (63.9–65.1%).

Two species from the *Nectandra* genus can be found at the northeast of Argentina: *N. angustifolia* and *N. megapotamica* (1). Both species are locally known as laurel amarillo, but only *N. angustifolia* is used in folk medicine for snake venom treatment (9).

To date, only *N. angustifolia* has been scientifically investigated with the active components isolated and characterized both structurally and functionally (9, 10). Recently Ricciardi et al. (11) presented preliminary results on the ability of some extracts from *N. megapotamica* to inhibit the coagulant activity of the *Bothrops diporus* (*yarará chica*) venom. The results obtained provoked us to evaluate the potential inhibition of the coagulant, hemolytic and proteolytic activity of the *yarará chica* venom by the aqueous and alcoholic extracts, and the essential oil of *N. megapotamica*.

Furthermore, as the oil was also evaluated, and in order to better characterize the chemical taxonomy of the population studied, we compared the chemical composition of *N. megapotamica* essential oil of Corrientes (Argentina) with that reported by Romoff et al. (8) for Brazil.

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Experimental

Venom sample

The venom sample was obtained by the usual milking method (12) from locally caught *B. diporus*. The pooled venom was dried at a reduced pressure and stored in a sealed flask at −15°C for further use. Prior to use it was reconstituted with physiological solution. Venom concentration was expressed in terms of dry weight.

Plant material and isolation of volatile constituents

In consideration of the possible variation in the chemical characteristics of a plant in response to seasonal factors, three samples of fresh leaves of *N. megapotamica*, representing the entire population, were collected randomly from Loma de Vallejos (Corrientes, Argentina, placed at 27,44°S latitude, 57,55°W longitude) during three different growth stages (I, autumn; II, spring; III, summer). The samples were representative of the species and its geographic area of distribution, and were chosen in order to be representative of the same pedoclimatic and collection conditions; the extraction conditions were also identical for all samples. Therefore, the influence of environmental and technical parameters on the chemical composition of the essential oil was avoided. The plants were identified by Prof. Tressens (IBONE/UNNE) and voucher specimens were deposited at the Herbarium of the IBONE (CTES 7104). The oils were obtained from leaves, previously dried during three days at a controlled temperature, by hydrodistillation using a macro distillation apparatus equipped with a 2-L flask. The isolation procedure was continued until the material was exhausted (4 hours). The oils were then dried with anhydrous sodium sulfate and kept in sealed flasks at −15°C until analysis. The distillation waters were also extracted with ethyl ether to provide the corresponding water oils enriched in oxygenated compounds.

Plant extracts

The *N. megapotamica* air-dried leaves were powdered, sieved and extracted by maceration with distilled water (24 hours), ethanol 95° (48 hours) or hexane (48 hours). The extracts were kept in desiccators under reduced pressure until further use (13). The extract concentrations, expressed as dry weight, were 26.2% (w/w), 8.7% (w/w) and 2.9% (w/w) for the water, ethanol and hexane extracts, respectively. Extracts were kept in a refrigerator in well-sealed containers until use.

Analysis of the essential oils

The components of the oil were analyzed as previously reported (14) and identified by comparison of their linear retention indices (LRIs) on two columns, determined in relation to a homologous series of *n*-alkanes (C_9–C_{26}) with those from pure standards or reported in the literature (15, 16). Comparison of fragmentation patterns in the mass spectra with those stored on the gas chromatography–mass spectrometry (GC–MS) database was also performed (17, 18).

SDS–PAGE analysis

The protein composition of snake venom was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using Mini-Protean II Electrophoresis Cell equipment. SDS–PAGE (10% w/v) in buffer, pH 8.8 (Tris 18.2%, SDS 0.4%), was performed on a slab according to the method of Laemmli (19), with 4% (w/v) stacking gel (buffer gel pH 6.8: Tris 6%, SDS 0.4%). The solutions for resolving gels and stacking gels for Tris–glycine–SDS–PAGE were

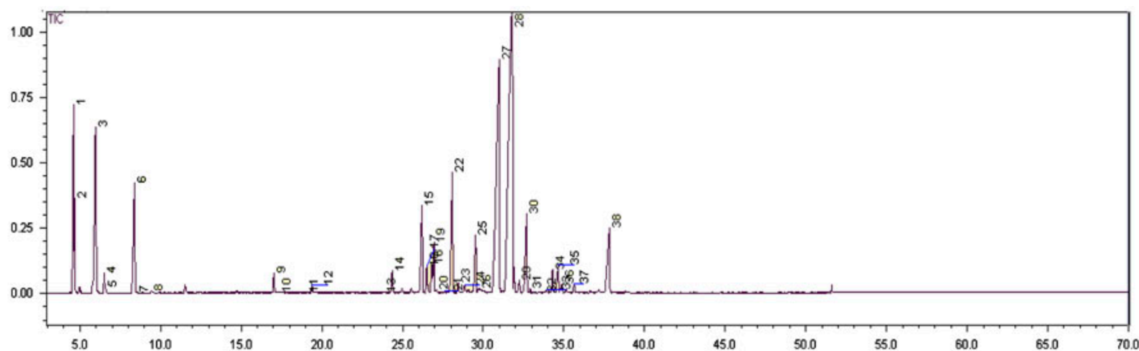

Figure 1. A typical total ion chromatogram of *Nectandra megapotamica* essential oil obtained from autumn vegetal material. *The peak numbers correspond to those from Table 1.

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Table 1. Composition (%) of the essential oil of *Nectandra megapotamica* from Argentina.

Peak number[a]	LRI SE 52[b]	LRI reported[c]	Compound[d]	%[e]			
				I[f] (autumn)	II (spring)	III (summer)	Water oil
1	933	932	α-Pinene	9.8	12.9	9.0	0.5-1.8
2	943	946	Camphene	0.2	0.3	0.2	0–0.1
3	976	974	β-Pinene	8.4	10.6	6.8	0.1-2.1
4	983	969	Sabinene	0.1	0.8	0.1	0–0.1
5	1013	988	β-Myrcene	0.6	0.1	0.5	0.1–0.2
6	1029	1024	Limonene	5.3	6.6	4.1	0.1-1.9
7	1030	1026	1,8-Cineole	–	–	–	0.8-3.0
8	1046	1032	(*Z*)-β-Ocimene	0.1	0.1	0.1	0–0.1
9	1085	1085	*p*-Mentha-2,4(8)-diene	0.3	0.3	0.2	0–0.2
10	1086	1095	Linalool	–	–	–	0–0.2
11	1184	1174	Terpinen-4-ol	–	–	–	0–0.2
12	1185	1184	*cis*-3-Hexenyl butanoate	0.3	0.1	0.1	0.1–0.4
13	1234	1229	*cis*-3-Hexenyl-2-methyl butanoate	0.1	0.1	0.1	0–0.1
14	1321	1346	Terpinyl acetate	0.3	–	0.1	0-2.7
15	1325	1335	δ-Elemene	4.3	0.3	0.3	0–0.2
16	1337	1345	α-Cubebene	0.2	4.3	4.7	0.1-2.1
17	1364	1373	α-Ylangene	0.2	–	–	0–0.1
18	1375	1374	α-Copaene	3.1	–	–	0-1.9
19	1383	1387	β-Bourbonene	0.9	0.1	0.1	0.2-1.1
20	1391	1387	β-Cubebene	2.6	2.4	0.1	0.2–0.4
21	1391	1389	β- Elemene	0.1	–	–	0-1.2
22	1421	1417	β-Caryophyllene	4.7	4.4	4.8	1.6-3.0
23	1439	1430	β-Copaene	0.5	0.4	–	0.2–0.3
24	1451	1439	Aromadendrene	0.3	0.2	–	0–0.3
25	1469	1452	α-Humulene	2.1	1.9	1.9	0.7-1.3
26	1475	1457	Rotundene	0.5	–	–	0–0.3
27	1484	1484	Germacrene D	17.8	16.9	18.5	6.2-10.4
28	1500	1500	Bicyclogermacrene	26.9	24.6	27.9	10.1-16.4
29	1521	1508	Germacrene A	0.6	0.1	0.4	0.1–0.2
30	1521	1522	δ-Cadinene	3.5	2.9	3.1	1.5-2.4
31	1547	1533	(*E*)-cadina-1,4-diene	0.2	–	0.1	0–0.1
32	1550	1537	α-Cadinene	0.2	–	0.2	0–0.1
33	1570	1555	Elemicin	0.2	–	0.1	0-1.5
34	1574	1561	(*E*)-Nerolidol	0.5	0.5	0.2	0.5-1.5
35	1587	1577	Spathulenol	0.9	0.1	0.4	0.2-1.6
36	1600	1592	Viridiflorol	0.3	0.1	–	0.1–0.5
37	1603	1616	(*Z*)-Asarone	0.1	0.1	0.1	2.1-4.2
38	1676	1675	(*E*)-Asarone	2.8	4.4	8.8	37.4-55.7
			Total	99.0	95.6	93.0	
			Grouped compounds				
			Monoterpene hydrocarbons	24.8	31.7	21.0	
			Oxygenated monoterpenes	0.3	0.0	0.1	
			Sesquiterpene hydrocarbons	68.9	58.5	62.2	
			Oxygenated sesquiterpenes	4.6	5.2	9.5	
			Other compounds	0.4	0.2	0.2	

Note: [a]Peak numbers correspond to the elution order in the total ion chromatogram (Figure 1).
[b]The components are reported according their elution order on SE-52.
[c]Linear retention indices (LRI) values from literature.
[d]Peak identifications are based on comparison of LRI values on two columns with those from pure standards or reported in the literature, and on comparison of mass spectra with file spectra.
[e]Relative proportions of the essential oil constituents were expressed as percentages obtained by peak-area normalization, all relative response factors being taken as one.
[f]For each compound reported, the values were not significantly different between samples ($p<0.05$).

prepared as previously reported (20). Gels were stained for 3–4 hours at room temperature with 0.25% (w/v) Coomasie brilliant blue R in 9.2% (v/v) acetic acid and 55.4% (v/v) methanol, and destained for 24 hours with several changes of 7% acetic acid and 30% (v/v) methanol (21).

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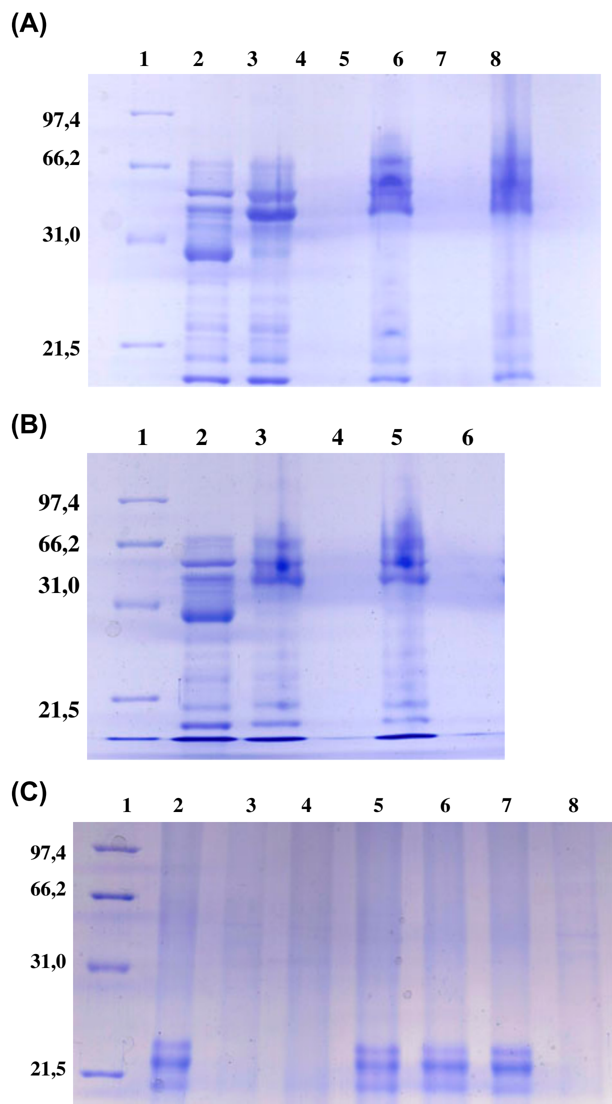

Figure 2. (A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of aqueous, alcoholic and hexanic leaf extracts. **1**: test (molecular weight pattern), **2**: *yarará* venom, **3**: venom + aqueous extract, **4**: aqueous extract, **5**, venom + alcoholic extract, **6**: alcoholic extract, **7**: venom + hexanic extract, **8**: hexanic extract. (B) SDS–PAGE of essential oil and distillation water oils. **1**: test (molecular weight pattern), **2**: *yarará* venom, **3**: venom + essential oil, **4**: essential oil, **5**, venom + water essence, **6**: water essence. (C) Neutralization of the proteolytic activity of venom by aqueous and alcoholic summer extracts. **1**: test (molecular weight pattern), **2**: casein pattern, **3**: venom + casein, **4**: aqueous extract + venom + casein, **5**, aqueous extract + casein, **6**: venom + alcoholic extract + casein, **7**: alcoholic extract + casein, **8**: venom + casein.

Neutralization of indirect hemolytic activity

Neutralization of *B. neuwiedi* venom enzymes by plant extracts and essential oils was measured using an indirect hemolytic assay on agarose–erythrocyte–egg yolk gel plate to define the minimum indirect hemolytic dose (MIHD) (22, 23). The MIHD of *B. diporus* venom was that which induced a hemolysis halo having a diameter of 10 mm after incubation for 18 hours at 37°C. Several plant extract:venom ratios were tested after being pre-incubated for 30 minutes at 37°C. Determinations were performed by quadruplicate and MIHD values expressed as the mean value corresponding to each extract.

Neutralization of coagulant activity

A minimum coagulant dose (MCD) was defined as the amount of *Bothrops* venom, which clots 0.2 mL plasma in 60 seconds (24). Coagulant activity was expressed by the normal coagulation time restitution percentage after addition of extract/oil induced by the snake venoms in the absence and presence of plant extracts.

Neutralization of proteolytic activity

The neutralization of the proteolytic activity of *B. diporus* (*yarará chica*) venom was performed following an adaptation of the SDS–PAGE technique (25, 26). Acrylamide solutions, gel buffer, stacking buffer and electrode buffer were as previously described for SDS–PAGE with 10% separation gel and 4% stacking. Casein mother solution contained 1 g of in 100 mL of buffer Tris–HCl 100 mM, pH 8. Buffer sample solution was concentrated twice, adding 4g of urea to improve results.

Sample solutions:

- casein, 50 μL from a casein solution (5 mg/mL) mixed with 50 μL of sample buffer;
- venom + casein, 50 μL of casein solution (10 mg/mL) incubated 60 minutes at 37°C with 50 μL of venom solution (0.125 mg/mL). The supernatant was later mixed with 50 μL of sample buffer;
- venom + extract + casein, 50 μL of venom solution (0.25 mg/mL) incubated 60 minutes at 37°C with 50 μL of extract solution (20 mg/mL). The supernatant was then incubated 60 minutes at 37°C with 50 μL of casein solution (10 mg/mL). Then 50 μL of sample buffer was added;
- extract + casein, 50 μL from a casein solution (10 mg/mL) incubated 60 minutes at 37°C with 50 μL of extract solution (10 mg/ml). The supernatant was then mixed with 50 μL of sample buffer.

All the samples were heated in a boiling water bath during 5 minutes for protein denaturation.

Table 2. Screening of the hemolytic, coagulant and proteolytic activities of the aqueous and alcoholic *Nectandra megapotamica* leaf extracts and oils.

	Neutralization of hemolytic activity		Neutralization of coagulant activity		Neutralization of proteolytic activity	
Extract	VE	HA	VE	Recovery %	VE	PAI
Autumn						
Aqueous	1:20	Inhibits (25%)	1:50	6	1:120	No
Alcoholic	1:20	No	1:50	10	1:120	Yes (<100%)
Hexanic	1:20	No	1:50	26	1:120	Yes (<100%)
Essential oil	1:40	Inhibits (25%)	1:150	26	1:220	Yes
Distillation water essence	1:40	No	1:150	74	1:220	No
Spring						
Aqueous	1:20	No	1:50	2	1:120	No
Alcoholic	1:20	No	1:50	–	1:120	Yes (<100%)
Hexanic	1:20	No	1:50	40	1:120	Yes (only a little)
Essential oil	1:40	No	1:500	28	1:400	Yes
Distillation water essence	1:40	No	1:500	82	1:400	Yes
Summer						
Aqueous	1:20	Inhibits (24%)	1:50	22	1:120	No
Alcoholic	1:20	No	1:50	6	1:120	Yes
Hexanic	1:20	Inhibits (16%)	1:50	7	1:120	Yes (<100%)
Essential oil	1:40	Inhibits (12%)	1:500	14	1:400	Yes
Distillation water essence	1:40	No	1:500	–	1:400	Yes

Note: VE, ratio venom:extract; HA, hemolytic activity; PAI, inhibition of the proteolytic activity.

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Results and discussion

Figure 1 presents a total ion chromatogram of *N. megapotapica* oil obtained from plant material collected in the autumn, while Table 1 shows the identified compounds in the volatile oil and their average percentages. For each oil sample, and the average values found for ethereal extract from water condensate (water oil), the retention index and the peak area percentages were calculated as mean values of two injections. The yields, calculated on basis of weight of fresh leaves, were determined as 0.11–0.18% (v/w).

The essential oils were characterized by high percentages of monoterpene and sesquiterpene hydrocarbon fractions varying on the amounts according to the seasonal stage. Oxygenated mono- and sesquiterpenes were found only in small amounts.

The compositions exhibited a completely different composition when compared with that previously reported (8), indicating the potential existence of chemical types within the species.

In water oil, oxygenated sesquiterpene hydrocarbons represented the most important fraction, (*E*)-asarone being the main compound (37.4–55.7%) followed by bicyclogermacrene (10.1–16.4%). In the leaf essential oils, the main compounds corresponded to the hydrocarbons fractions (mono- and sesquiterpenes): bicyclogermacrene, germacrene D and α-pinene. Less represented were oxygenated sesquiterpenes, with (*E*)-asarone the main component.

Figure 2(A) shows the aqueous extract produced a modification on the venom profile, especially a decrease on intensity of the 28-kDa band (trombine type protein) and increasing the 40-kDa intensity band. Aqueous and hexanic extracts exhibited higher interaction with venom, provoking the disappearing of 28-kDa band, and the simultaneous decreasing of the 18- and 16-kDa bands, coincident with those of the phospholipases. This interaction was similar to that produced by the essential and distillation water oils as shown in Figure 2(B).

The aqueous extract and the essential oil inhibited up 25% of the hemolytic activity of venom and this occurred mainly in the autumn (Table 2): during summer the activity diminished and in the spring there was no activity.

The hexanic extract and water oil showed the major activity neutralizing the *yarará* procoagulant venom in the autumn and spring.

Alcoholic extracts and essential oils were active neutralizing the proteolytic activity of *yarará chica* venom.

The visualization of casein band indicated inhibition of proteolytic venom activity of the extract/oil. Figure 2(C) shows the electrophoretic behavior of the interaction between extracts/oils and venom/casein, avoiding interferences with vegetable proteases in the data interpretation; the casein + extract/oil interaction was also evaluated by electrophoresis.

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On the basis of the chemical information obtained, and according to the evaluation of the species behavior in response to seasonal factors over the year, we observed that we could discard the seasonal effect on the chemical composition of *N. megapotamica*. In addition, characterization of the oil compounds revealed chemical markers, which can assist in defining the profile of the species growing wild in Argentina.

These results clearly differ from that obtained by Romoff et al. (8) from the São Paulo samples, opening the option for the existence of chemical types, which should be considered for future applications of the oils.

The results obtained in the evaluation of the activity of *N. megapotamica*, extracts against *yarará chica* venom showed *in vitro* activity for the neutralization of the hemolytic action (aqueous extract and direct essential oil), procoagulant (hexanic extract and distillation water essence) and proteolytic activity (alcoholic extract and distillation water essence).

These activities were not as interesting, if focused in their applications, when compared with those obtained for the *N. angustifolia* extracts (9). However, the results should alert us about the botanical identification and the selection of more convenient vegetative stage to collect the plants.

Pharmacological studies need to be performed using new extract fractions in order to isolate and characterize the active principle responsible for the antiophidian activity.

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References

1. J.G. Rohwer, *Lauraceae: Nectandra*. Flora Neotropica Monogr., **60**, 1–332 (1993).
2. H. Richter and M. Dallwitz, *Maderas comerciales*. (http://delta-intkey.com/wood/es/www/launemeg.htm) (9 February 2013).
3. C. Marqués, *Importancia econômica da familia Lauraceae Lindl*. Floresta e Ambiente, **8**, 195–206 (2001).
4. A. Da Silva Filho, M. Andrade e Silva, J. Carvalho and J. Bastos, *Evaluation of analgesic and anti-inflammatory activities of* Nectandra megapotamica *(Lauraceae) in mice and rats*. J. Pharm. Pharmacol., **56**, 1179–1184. (2004a).
5. Da Silva Filho, S. Albuquerque, M. Silva, M. Eberlin, D. Tomazela and J. Bastos, *Tetrahydrofuran lignans from* Nectandra megapotamica *with trypanocidal activity*. J. Nat. Prod., **67**, 42–45 (2004b).
6. Da Silva Filho, E. Costa, W. Cunha, M. Silva, N. Nanayakkara and J. Bastos, In vitro *antileishmanial and antimalarial activities of tetrahydrofuran lignans isolated from* Nectandra megapotamica *(Lauraceae)*. Phytother. Res., **22**, 1307–1310 (2007).
7. M.A. Apel, M.E. Lima, A. Souza, I. Cordeiro, M.C. Young, M. Sobral, I.B. Suffredini and P.R. Moreno, *Screening of the biological activity from essential oils of native species from the Atlantic rain forest (Sao Paulo – Brazil)*. Pharmacol. Online, **3**, 376–383 (2006).
8. P. Romoff, M. Ferreira, R. Padilla, D. Toyama, O. Fávero and J. Lago, *Chemical composition of volatile oils from leaves of* Nectandra megapotamica *Spreng. (Lauraceae)*. Química Nova, **33**, 1119–1121 (2010).
9. A. Torres, F. Camargo, G. Ricciardi, E. Dellacassa and A. Ricciardi, *Neutralizing effects of* Nectandra angustifolia *extracts against* Bothrops neuwiedi snake venom. Nat. Prod. Comm., **6**, 1393–1396 (2011).
10. A.M. Torres *Chemotaxonomic Characterisation of Different* Nectandra *Species from the Northeast of Argentina. Evaluation of their Activity Against* Bothrops diporus *Venom (COPE) 'Yarará chica'*. Ph.D. thesis, Universidad Nacional del Nordeste, Corrientes, Argentina (2011).
11. G.A.L Ricciardi, A.M. Torres, F.J. Camargo, A.E. Agrelo de Nassiff, E. Dellacassa and A.I.A. Ricciardi. *Caracterización quimiotaxonómica y evaluación de la actividad contra veneno de yarará de* Nectandra megapotámica *(Spreng.) Mez*. XVII Congreso Ítalo-Latinoamericano de Etnomedicina 'Bernardino d' Ucria' SILAE, Palermo, Italia (2008).
12. R.G. Markfarlan, *Russell's viper venoms, 1953–1964*. Bri. J. Haematol., **13**, 437–451 (1967).
13. R. Otero, V. Núñez, J. Barona, R. Fonnegra, S. Jimenez, R. Osorio, M. Saldarriaga and A. Díaz, *Snakebites and ethnobotany in the northwest región of Colombia Part. III Neutralization of haemorragic effect of* Bothrops atrox *venom*. J. Ethnopharmacol., **73**, 233–241 (2000).
14. D. Lorenzo, I. Loayza and E. Dellacassa, *Composition and chiral characterization of the essential oil of* Buddleja tucumanensis *from Bolivia*. Flavour Fragr. J., **21**, 95–98 (2006).
15. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th edn. Allured Publ. Corp., Carol Stream, IL (2007).
16. N.W. Davies, *Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases*. J. Chromat., **503**, 1–24 (1990).
17. F.W. McLafferty and D.B. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, 5th edn. Wiley, New York, NY (1991).
18. M. Dávila, I. Loayza, D. Lorenzo and E. Dellacassa, *Searching for natural bioactive compounds in four* Baccharis *species from Bolivia*. Nat. Prod. Commun., **3**, 1–6 (2008).
19. U.K. Laemmli, *Cleavage of structural proteins during the assembly of the head of bacteriophage T4*. Nature, **227**, 680–685 (1970).
20. A.M. Pilosof and G.B. Bartholomai, *Caracterización funcional y estructural de proteínas*, pp. 159–166, CYTED-Eudeba Universidad de Buenos Aires, Buenos Aires (2000).
21. F. Camargo, A. Torres, G. Ricciardi, A. Ricciardi and E. Dellacassa, *SDS PAGE: A useful tool for preliminary screening of antisnake activity of plant extracts*. B.L.A.C.P.M.A., **10**, 429–434 (2011).
22. J. Gutierrez, C. Avila, E. Rojas and L. Cerdas, *An alternative* in vitro *method for testing the potency of the polyvalent antivenom produced in Costa Rica*. Toxicon, **26**, 411–413 (1988).

23. R. Otero, V. Nuñez, R. Osorio, J. Gutiérrez, C. Giraldo and L. Posada, *Ability of six Latin American antivenoms to neutralize the venom of mapana equis (*Bothrops atrox*) from Antioquia and Chocó (Colombia)*. Toxicon, **33**, 809–815 (1995).
24. J. Gene, A. Roy, G. Rojas, J. Gutiérrez and L. Cerdas, *Comparative study on coagulant, defibrinating, fibrinolytic and fibrinogenolytic activities of Costa Rican crotaline snake venoms and their neutralization by a polyvalent antivenom*. Toxicon, **27**, 841–848 (1989).
25. M. Pardo and C. Natalucci, *Electrophoretic analysis (tricine–SDS–PAGE) of bovine caseins*. Acta Farm. Bonaerense, **21**, 57–60 (2002).
26. Gay, L. Leiva, R. Ruiz and O. Acosta, *Inhibición de la actividad proteolítica del veneno de* Bothrops alternatus *por quelantes de metales*. Comunicaciones Científicas y Tecnológicas UNNE, E-015 (2004).

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