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Short report

Bioactive plants from Argentina and Bolivia

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Abstract

Antibacterial and molluscicidal activities of methanol and chloroform extracts of 16 plant species belonging to the families Compositae and Melastomataceae were evaluated. The chloroform extract of *Vernonanthura tweediana* and the methanol extract of *Senecio santelisis* resulted to be very toxic to brine shrimp nauplii (LC_{50}=1 μg/ml). Chloroform extracts of *S. santelisis* and *Senecio leucostachys* as well as the methanol extract of *Wedelia subvaginata* displayed molluscicidal effects on *Biomphalaria peregrina* showing LC_{100}<100 μg/ml. Moderate antibacterial action was produced by the chloroform extracts of *Flaveria bidentis*, *Grindelia scorzonerifolia* and *Vernonia incana* against two strains of *Staphylococcus aureus*.

Keywords: Compositae; Melastomataceae; Antibacterial activity; Molluscicidal activity; Brine shrimp test

1. Plants

Species, collection sites and dates, are listed in Table 1. Vouchers are on deposit in the Herbarium of the Fundación Miguel Lillo, Tucumán, Argentina.

2. Uses in traditional medicine

Extracts of *Miconia* spp. possess analgesic effects [1,2], however, there are no reports on the use of *M. calvescens* in traditional medicine. *Senecio puchii* is used in the treatment of stomachache and to lower blood pressure [3] and *Flaveria bidentis* as digestive, anthelmintic, stimulative and in menstrual troubles [4]. *Siegesbeckia* spp. are used for wounds and burns, to treat rheumatism, renal colic, syphilis, leprosy and various skin diseases [5]. Many Vernonieae spp. have been used in the treatment of mycosis, dysentery, ulcers, syphilis, and parasites [6,7].

The aqueous extract of *Vernonia scorpioides* has been employed for the treatment of allergies, skin parasites, skin injuries and itching [8,9]. *Wedelia paludosa*, a Brazilian medicinal plant has been employed in folk

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Table 1
Bioactive plants from Argentina and Bolivia

Plants	Tribe	Family	Collection date	Collection site
Chrysolaena flexuosa Sims.	Vernonieae	Asteraceae	December 1997	Itá Ibaté, Corrientes, Argentina
Dinoseris salicifolia Griseb.	Mutisieae	Asteraceae	February 1999	Canasmoro, Tarija, Bolivia
Flaveria bidentis L.	Helenieae	Asteraceae	February 1999	El Alto, Tarija, Bolivia
Grindelia scorzonerifolia Hook. et Arn.	Astereae	Asteraceae	January 2000	Añatuya, Santiago del Estero, Argentina
Miconia calvescens D.C.	Miconieae	Melastomataceae	February 1999	Bermejo, Bolivia
Senecio puchii Phil.	Senecioneae	Asteraceae	January 2000	Paso de Jama, Jujuy, Argentina
Senecio santelisis Phil.	Senecioneae	Asteraceae	January 2000	Susques, Jujuy, Argentina
Senecio leucostachys Baker	Senecioneae	Asteraceae	April 2000	Anillaco, La Rioja, Argentina
Sigesbeckia jorullensis H. B. K.	Heliantheae	Asteraceae	March 2000	Colonia Benítez, Chaco, Argentina
Urmenetea atacamensis Phil.	Mutisieae	Asteraceae	April 2002	Antofagasta de la Sierra, Catamarca, Argentina
Vernonanthura squamulosa Hook. et Arn.	Vernonieae	Asteraceae	September 2000	Horco Molle, Tucumán, Argentina
Vernonanthura tweediana Baker	Vernonieae	Asteraceae	December 1999	Aristóbulo del Valle, Misiones, Argentina
Vernonia cognata Less.	Vernonieae	Asteraceae	December 1999	Paraná, Entre Ríos, Argentina
Vernonia scorpioides Lam.	Vernonieae	Asteraceae	November 2000	Horco Molle, Tucumán, Argentina
Wedelia subvaginata N. E. Br.	Heliantheae	Asteraceae	December 1999	Clorinda, Formosa, Argentina

medicine as analgesic [10] and its trypanosomicidal activity has been reported [11]. The *Wedelia calendulacea* alcoholic extract has shown hepatoprotective effects [12]. *Urmenetea atacamensis* has been used as a "coca" (*Eritroxylum coca*) substitute [13]. Mild antispasmodic, expectorant and hypotensive effects have been reported from some *Grindelia* spp. [14]. Finally, there are no reports on the use of *Dinoseris salicifolia* in traditional medicine.

3. Previously isolated classes of constituents

Miconia: benzoquinones and flavonoids [15]. *Senecio*: pirrolizidinic alkaloids [16]. *Flaveria*: flavonoids [17]. *Grindelia* and *Sigesbeckia*: diterpenoids [18–20]. *Chrysolaena*: sesquiterpene lactones, lignanes and flavonoids [21]. *Vernonanthura*: diterpenes [22], sesquiterpene lactones [23]. *Vernonia*: sesquiterpene lactones [24–26]. *Wedelia*: diterpenes [11,12]. *Urmenetea*: triterpenoids [27]. *Dinoseris*: sesquiterpene lactones [28].

4. Tested material

Successive $CHCl_3$ and MeOH extracts were obtained from ground air-dried aerial parts with the yields described in Table 2.

5. Studied activity

General toxicity to brine shrimp [29], antibacterial [30,31], and molluscicidal activity [32,33].

6. Used organisms and bioassays

6.1. Antibacterial activity

Lactobacilli listed in Table 2 were obtained from Centro de Referencia para Lactobacilos (CERELA), Tucumán, Argentina. *Staphylococcus aureus* ATCC 6538 P, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 39212 were provided by ATCC. *Klebsiella pneumoniae* F 350 and *S. aureus* F 7 were obtained from the Microbiology Laboratory of Hospital Nicolás Avellaneda, Tucumán, Argentina.

The antibacterial test was carried out using the agar diffusion technique and the diameters of inhibition zones were measured in millimeters. The MIC values of extracts were determined by the microdilution method in Müller–Hinton medium [31]. MBC values were determined by plating out aliquots of 0.01 ml (undiluted) of the MIC testing samples showing no growth onto MH agar plates.

Table 2
Bioactivity of plant extracts from Argentina and Bolivia

Plant	Plant part	Solvent	Yield %	Bioassays								
				Biomphalaria peregrina LD_{50} (μg/ml)	BST LD_{50} (μg/ml)	Inhibition zone (mm)						
						A	B	C	D	E	F	G
Chrysolaena flexuosa	L+F	$CHCl_3$	4.9	>100	>1000	12	6	–	–	–	–	–
	L+F	MeOH	1.9	>100	326	7	12	6	–	–	–	12
Dinoseris salicifolia	L+F	$CHCl_3$	7.7	>100	46	8	8	–	–	–	–	–
	L+F	MeOH	7.9	>100	>1000	–	–	–	–	–	–	20
Flaveria bidentis	L+F	$CHCl_3$	1.7	>100	ND	10	16	–	–	–	–	5
	L+F	MeOH	2.1	>100	137	7	8	–	–	–	–	10
Grindelia scorzonerifolia	L+F	$CHCl_3$	3.2	>100	140	14	16	–	–	–	–	12
	L+F	MeOH	7.0	>100	76	–	–	–	–	–	–	10
Miconia calvescens	L	$CHCl_3$	1.7	>100	5	–	7	–	–	–	–	–
	L	MeOH	1.1	>100	>1000	–	–	–	–	–	–	–
Senecio puchii	L+F	$CHCl_3$	9.0	>100	109	–	–	–	–	–	–	20
	L+F	MeOH	8.7	>100	202	9	10	7	–	–	–	12
Senecio santelisis	L+F	$CHCl_3$	5.7	50	49	10	8	–	–	–	–	20
	L+F	MeOH	3.9	>100	1	11	11	12	–	7	7	18
Senecio leucostachys	L	$CHCl_3$	9.0	85	>1000	8	6	6	–	–	–	10
	L	MeOH	8.5	>100	>1000	–	–	–	–	–	–	5
Sigesbeckia jorullensis	L+F	$CHCl_3$	2.8	>100	196	13	10	15	–	–	15	15
	L+F	MeOH	6.3	>100	112	7	8	10	7	–	–	10
Urmenetea atacamensis	L	$CHCl_3$	1.2	>100	>1000	–	12	–	–	–	–	–
	L	MeOH	3.0	>100	>1000	7	10	–	–	–	–	14
	F	$CHCl_3$	1.4	>100	>1000	12	12	–	–	–	–	–
	F	MeOH	4.3	>100	>1000	–	–	–	–	–	–	–
Vernonanthura squamulosa	L	$CHCl_3$	10.8	>100	>1000	13	11	7	–	–	–	18
	L	MeOH	9.7	>100	>1000	–	–	–	–	–	–	–
	F	$CHCl_3$	3.5	>100	>1000	9	8	7	–	–	–	15
	F	MeOH	6.0	>100	>1000	–	6	–	–	–	–	–
Vernonanthura tweediana	L+F	$CHCl_3$	10.2	>100	1	–	–	–	–	–	–	–
	L+F	MeOH	3.4	>100	ND	7	12	–	–	–	–	9
Vernonia cognata	L+F	$CHCl_3$	6.9	>100	>1000	12	8	–	–	–	–	16
Vernonia incana	L+F	$CHCl_3$	9.4	>100	>1000	16	15	–	–	–	–	14
	L+F	MeOH	2.4	>100	>1000	7	10	–	–	–	–	12
Vernonia scorpioides	L	$CHCl_3$	3.4	>100	>1000	11	8	6	–	–	–	10
	L	MeOH	5.4	>100	>1000	–	–	–	–	–	–	–
Wedelia subvaginata	L+F	$CHCl_3$	10.3	100	>1000	8	10	–	–	–	–	10
	L+F	MeOH	7.5	81	136	7	7	7	7	–	–	12
Penicillin 10 U						10	–	20	30	30	18	–

ND: not detected. L: leaves; F: flowers; – : no inhibition zone. A: *Staphylococcus aureus* ATCC 6538 P; B: *Staphylococcus aureus* clinically isolated; C: *Enterococcus faecalis* ATCC 39212; D: *Lactobacillus paracasei* ssp. *paracasei* CE 75; E: *Lactobacillus plantarum* CE 105; F: *Lactobacillus plantarum* CE 358; G: *Lactobacillus acidophilus* ATCC 521. Extracts concentration per hole: 1 mg/20 μl EtOH.

6.2. Molluscicidal activity

Biomphalaria peregrina adults were collected from small lakes in Tucumán province, Argentina and maintained in an aquarium with distilled water, mineralized with 0.05 g/l of $Ca_3(PO_4)_2$ at 26±2 °C, pH 7.2 and fed on *Lactuca sativa* fresh leaves. Aquarium snails, uniform in diameter of the shell (7 mm) and age, were selected for the bioassay. Snails were maintained for 24 h without feeding before the experiment. Extracts (10 mg) were dissolved in 2 ml of MeOH and diluted with water in order to obtain concentrations of 100, 50 and 25 μg/ml. Five snails were used for each concentration. In control experiments five snails were introduced in water:MeOH 98:2, 99:1, and 99.5:0.5. MeOH was not toxic for the snails at the concentrations tested. After an exposure of 24 h, snails were placed in a Petri dish and the heartbeat detected upon microscopic observation. After the exposure period, alive snails were returned to fresh water and controlled for another 24 h in order to check movement and register mortality. Snails, which survived, were not

Table 3
MIC and MBC (μg/ml) of plant extracts from Argentina and Bolivia

Plants	Plant part extracted	Solvent	Microorganisms					
			A		B		C	
			MIC	MBC	MIC	MBC	MIC	MBC
Vernonanthura squamulosa	L	$CHCl_3$	2500	2500	1250	2500	>2500	>2500
Chrysolaena flexuosa	L+F	$CHCl_3$	2500	–	2500	–	>2500	>2500
Vernonia cognata	L+F	$CHCl_3$	2500	–	1250	–	>2500	>2500
Vernonia incana	L+F	$CHCl_3$	2500	–	625	–	>2500	>2500
Grindelia scorzonerifolia	L+F	$CHCl_3$	2500	–	1250	–	>2500	>2500
Vernonia scorpiodes	L	$CHCl_3$	1250	–	625	–	>2500	>2500
Sigesbeckia jorullensis	L+F	$CHCl_3$	625	2500	312.5	–	78	78

L: leaves; L+F: leaves and flowers; A: *Staphylococcus aureus* ATCC 6538 P; B: *Staphylococcus aureus* clinically isolated; C: *Enterococcus faecalis* ATCC 39212; (–): no bactericide at doses assayed.

subjected to further tests. A 10 μg/ml water solution of $CuSO_4$ was used as positive control [34]. Data were analyzed with the Finney computer program to determine the LD_{50} at a 95% confidence interval [35].

6.3. General toxicity

The tiny crustacean *A. salina* is used. When the eggs were placed in a brine solution, hatch occurred within 48 h. Just born nauplii moved towards artificial light sources. Immediately, ten nauplii were placed in vials containing the plant extracts dissolved in 5 ml of brine solution at concentrations of 1, 10, 100 and 1000 μg/ml. Survivors were counted after 24 h with the aid of a stereoscopic microscope and LD_{50} values at a 95% confidence limit were calculated. Experiments were conducted in three replicates [29].

7. Results

General toxicity, antibacterial on Gram (+) strains, and molluscicidal activities are reported in Table 2, and MIC and MBC values in Table 3. No antibacterial effects were produced by the thirty-five extracts on the Gram (−) strains *E. coli*, *P. aeruginosa* and *K. pneumoniae*.

8. Conclusion

Thirty-five extracts from sixteen plants native to the north of Argentina and south Bolivia were submitted to bioassays in order to evaluate toxicity against *A. salina*, antibacterial and molluscicidal effects.

As reported in Table 2, the most toxic extracts against *A. salina* were the chloroform extracts from *V. tweediana* (LD_{50}=1 ppm), *M. calvescens* (LD_{50}=5 ppm), *D. salicifolia* (LD_{50}=46 ppm) and *S. santelisis* (LD_{50}=49 ppm) and the MeOH extracts from *S. santelisis* (LD_{50}=1 ppm) and *G. scorzonerifolia* (LD_{50}=76 ppm).

The molluscicidal effects were evaluated against *B. peregrina* (Table 2). As shown, the extracts of *S. santelisis* (LD_{50}=50 ppm) and *S. leucostachys* (LD_{50}=85 ppm), as well as the MeOH extract from *W. subvaginata* (LD_{50}=81 ppm) were the most toxic. The former results indicated that there was no direct relationship between the effects on *A. salina* and the molluscicidal activity, since the very toxic extracts on *A. salina*, as the chloroform extract of *S. santelisis*, resulted non-toxic to the mollusc. On the other hand, *S. leucostachys* was very toxic for the mollusc but resulted innocuous for *A. salina*.

Finally, the antibacterial effects were reported in Tables 2 and 3. The *Staphylococci* strains as well as *L. acidophilus* ATCC 521 were inhibited to a certain extent by most of the tested extracts. Noteworthy, *Lactobacilli* strains from CERELA collection, which were isolated from natural environments of the north of Argentina were resistant to the majority of our extracts that also came from plants collected in the same area. These results were consistent with reports [36] that indicated that "natural environments are colonized by special microorganisms because during the colonization process only the most resistant can survive". The disk diffusion assay shows that the strongest antibacterial effects against both *S. aureus* strains were produced by the chloroform extracts from *F. bidentis*, *G. scorzonerifolia* and

V. incana. The remaining extracts displayed moderate, low or no antibacterial effects. Finally, MIC and MBC values (Table 3) obtained in liquid media, indicated no relevant antibacterial action on the three strains tested.

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References

[1] Vasconcelos MA, Ferreira DS, Andrade e Silva ML, Veneziani RC, Cunha WR. Boll Chim Farm 2003;142:333.
[2] Spessoto MA, Ferreira DS, Crotti AE, Silva ML, Cunha WR. Phytomedicine 2003;10:606.
[3] Villagran C, Romo M, Castro V. Chungará (Arica) 2003;35:73.
[4] Ratera EL, Ratera MO. Plantas de la Flora Argentina empleadas en Medicina popular. Buenos Aires: Hemisferio Sur S.A; 1982. p. 99.
[5] Botanical.com. Available on line at http://www.botanical.com/botanical/mgmh/s/sieges48.html.
[6] Rakotoarimanga JV, Krebs HC, Habermehi G, Rasoanaivo P, Ratsimamanga-Urverg S, Rakoto-Ratsimamanga A. Fitoterapia 1992;63:271.
[7] Rasoanaivo P, Petitjean A, Conan JY. Fitoterapia 1993;64:114.
[8] Moraes MD. A familia Asteraceae na planicie litoranea de Picinguaba, Master Thesis, Instituto de Biología, Universidade Estadual de Campinas, Sao Paulo, Brazil. 1997.
[9] Cabrera AL, Klein RM. Compostas: 3.Tribo Vernonieae. Flora Ilustrada Catarinense, vol. 354; 1980.
[10] Block LC, Scheidt C, Quintao NL, Santos AR, Cechinel-Filho V. Pharmazie 1998;53:716.
[11] Batista R, Chiari E, de Oliveira AB. Planta Med 1999;65:283.
[12] Mottakin AK, Chowdhury R, Haider MS, Rahman KM, Hasan CM, Rashid MA. Fitoterapia 2004;75:355.
[13] Victoria Castro R. Ayquina y Toconce: Paisajes Culturales del Norte Arido de Chile 2001 Proyecto Fondecyt 101100682001.
[14] Toursarkissian M. Plantas Medicinales de la Argentina. Buenos Aires: Hemisferio Sur S.A; 1980. p. 33.
[15] Gunatilaka AA, Berger JM, Evans R, Miller JS, Wisse JH, Neddermann KM, et al. J Nat Prod 2001;64:2.
[16] Macel L, Vrieling K, Klinkhamer PC. Phytochemistry 2004;65:865.
[17] Pereyra de Santiago OJ, Juliani HR. Experientia 1972;15:380.
[18] Ybarra MI, Borkosky SA, Bardón A, Asakawa Y. J Nat Prod 2005;68:554.
[19] Jolad SD, Timmermann BN, Hoffmann JJ, Bates RB, Siahaan TJ. Phytochemistry 1987;26:483.
[20] Xiang Y, Zhang H, Fan CQ, Yue JM. J Nat Prod 2004;67:1517.
[21] Bardón A, Montanaro S, Catalán CAN, Díaz JG, Herz W. Phytochemistry 1997;34:253.
[22] Borkosky S, Bardón A, Catalán CAN, Díaz JG, Herz W. Phytochemistry 1995;40:1477.
[23] Borkosky S, Bardón A, Catalán CAN, Díaz JG, Herz W. Phytochemistry 1997;44:465.
[24] Bardón A, Catalán CAN, Gutiérrez AB, Herz W. Phytochemistry 1988;27:2691.
[25] Bardón A, Catalán CAN, Gutiérrez AB, Herz W. Phytochemistry 1988;27:2989.
[26] Bardón A, Kamiya NI, Ponce de León CA, Catalán CAN, Díaz JG, Herz W. Phytochemistry 1992;31:609.
[27] Maldonado Z, Hoeneisen M, Silva MA. Phytochemistry 1988;27:2993.
[28] Pastoriza A, Gianfrancisco S, Riscala E. Rev Fac Agron (LUZ) 2000;17:261.
[29] Mc Laughlin JM. Crown gall tumors on potatoes discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractionation. In: Hostettmann K, Dey PM, Harborne JB, editors. Assays for bioactivity. San Diego: Academic Press; 1991. p. 2.
[30] Klaenhammer TR. Biochemie 1988;70:337.
[31] National Committee for Clinical Laboratory Standards (NCCLS). 5th ed. Pennsylvania: Wayne; 2000. Document M7-A5.
[32] World Health Organization. Monogr Ser 1965;50.
[33] Malek EA, Cheng TC. Medical and economic malacology. London: Academic Press; 1974. p. 299.
[34] Yoke Marchant Y, Balza F, Abeysekera BF, Towers GHN. Biochem Syst Ecol 1984;12:285.
[35] Finney DJ. Probit anal. Cambridge: University Press; 1971.
[36] Gonzalez S, Romero N, Apella C, Ruiz Holgado A, Oliver G. Microbiol Alim Nutr 1987;5:317.