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Screening of 239 Paraguayan plant species for allelopathic activity using the sandwich method

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ABSTRACT

We evaluated the allelopathic potential of 239 Paraguayan plants using the sandwich method. The samples were collected from 3-different regions of Paraguay. A total of 130 species, 47 families were collected from (i). Botanical Garden and Zoo of Asunción and its surroundings, (ii). 71 species (40 families) from Mbaracayú Natural Reserve and (iii). 38 species (25 families) from the Chaco region. We found the species with high inhibitory potential, such as *Cleome aculeata* (Cleomaceae), which completely inhibited the germination of lettuce. Others spp. strongly inhibited the growth of lettuce seedlings viz., *Strychnos brasiliensis* (Loganiaceae), *Pterogyne nitens* (Fabaceae), *Sorocea bonplandii* (Moraceae), *Rollinia emarginata* (Annonaceae), *Microstachys hispida* (Euphorbiaceae), *Prosopis ruscifolia* (Fabaceae) and *Senna* sp. (Fabaceae). These results demonstrated high allelopathic potential of Paraguayan plant species.

Key words: Allelopathy, *Cleome aculeata*, germination, lettuce, Paraguayan plants, *Pterogyne nitens,* sandwich method, seedling growth, *Sorocea bonplandii, Strychnos brasiliensis.*

INTRODUCTION

South America has rich biodiversity and is the centre of origin of several cultivated plants (19,47). Paraguay, also called "the heart of South America" due to its geographical location, also has rich flora and fauna (24,44). According to Vavilov's centers of origin of cultivated plants, Paraguay is catalogued as part of the Brazilian-Paraguayan Sub-Centre. A total of 13 species have originated in this area, including cassava (*Manihot esculenta)*, peanut (*Arachis hypogaea*), pineapple (*Ananas comosus*), stevia (*Stevia rebaudiana*) and yerba mate (*Ilex paraguariensis*) (47).

The concept (Phenomenon) of allelopathy has been reported from Roman times, but the term was coined by Hans Molisch in 1937. It refers to the phenomenon, where a plant

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releases chemical compounds (mostly secondary metabolites), in to the environment, which influences the growth and development of its neighbours in a positive (growth promoting) or negative (growth inhibition) way (37,38).

Recently allelopathy research has been done on allelopathic plants in South America, especially in Brazil (3,23,25,29,36) and Peru (27), but still many plants are not investigated. To date, almost no work has been done on allelopathic activity of Paraguayan plants. This research aimed to evaluate the allelopathic potential of 239 Paraguayan plants, using the sandwich method (7). This study will increase the knowledge about these native plants.

MATERIALS AND METHODS

Plant Materials

In March 2006 the plant samples were collected from 3-different regions; Central Department, Departments of Canindeyú, and Presidente Hayes.

(i). A total of 130 species (47, families) were collected from the Botanical Garden and Zoo, Asunción and its surroundings (Central Department). This area as per Köppen Climate Classification has humid sub-tropical climate with humid warm summer (Cfa). The annual mean temperature is around 22-23 °C, with an average high temperature of 28 °C and an average minimum temperature of 16 °C. The average annual rainfall is 1,400 mm, with a rainy season from October to April and dry season from June to August (10,20).

(ii). A total of 71 species (40 families) were collected from the Mbaracayú Natural Reserve (Department of Canindeyú). This reserve is part of Alto Paraná formation of Atlantic rainforest. It has sub-tropical climate of Mid Latitude with a dry winter (Cw). The annual mean rainfall: 1,800 mm in the rainy season (October to March). Temperature fluctuations mark seasonality, the mean daily low-high temperature is in range of 14-25 °C in July and 22-34 °C in January (5,13).

(iii). A total of 38 species (25 families) were collected from the Chaco region (Department of Presidente Hayes). This region extends from the tropics (tropical savanna climate, Aw) to the sub-tropics (sub-tropical climate, Cw). Summer rainfall is heaviest in east (mean 1,300 mm) and lowest in the west (mean only 400 mm). In summer season 80 % rain occur. Annual mean temperatures vary from 24 to 26 °C, with a maximum temperature of 44 °C and a minimum temperature of -5.1 °C (10,12,22).

From the above 3-locations, a total of 239 species (76 families) were collected (Fig. 1 and Table 1). The plant materials were oven dried at 60 °C for 24 h (HS 1000-I, Taiki Sangyo Co. Ltd.) and then stored in plastic bags until further use.

Of the collected species, Asteraceae and Fabaceae were the largest families represented with 27 species each, followed by Poaceae and Verbenaceae with 11 species each and Euphorbiaceae with 10 species. All the plants scientific names were corroborated in The Plant List (http://www.theplantlist.org/).

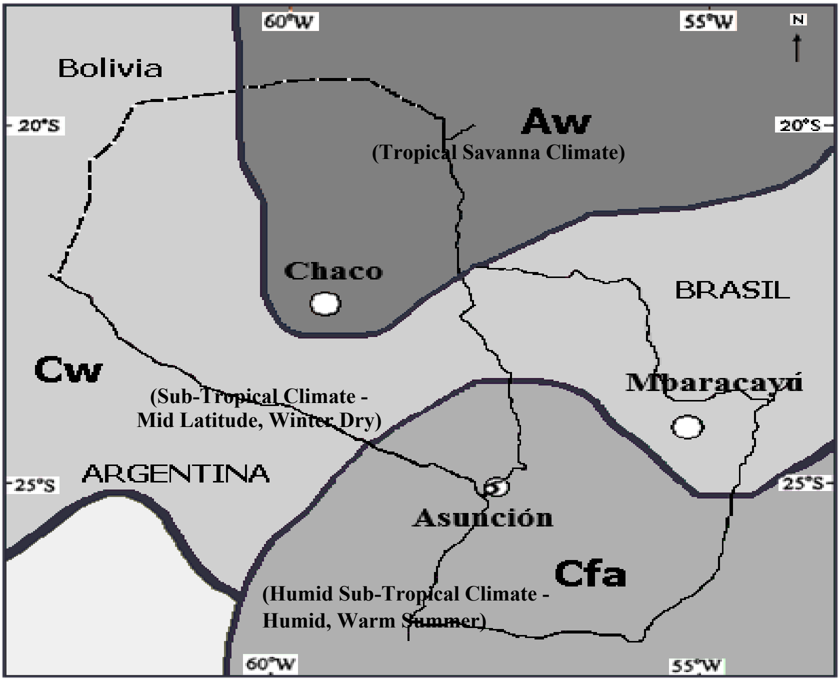

Figure 1**.** Map of Paraguay and collection sites with Köppen Climate classification

Table 1. Plant materials collection sites in Paraguay and nature of collected materials

Collection Sites	Climate	No. of Family	No. of Species		
			Herbaceous	Woody	Total
(i). Botanical Garden and Zoo Asunción and its surroundings	Cfa (Humid Sub-Tropical) Climate-Humid, Warm Summer	47	82	48	130
(ii). Mbaracayú Natural Reserve	Cw (Sub-Tropical Climate-Mid Latitude, Winter)	40	28	43	71
(iii). Chaco Region	Aw (Tropical Savanna Climate)	25	8	30	38
Total		76	118	121	239

Sandwich Method

The sandwich method (7) was adopted, as screening method to evaluate the allelopathic activity of leaf leachates from Paraguayan plants. It is fast and reliable method to elucidate the allelopathic activity from leaf leachates of large number of samples under laboratory conditions. Either 10 or 50 mg of dried leaves were placed in each well of a six-well multidish plastic plate (9.6 cm^2 area per well i.e. 35 mm dia and 18 mm depth; Nalge Nunc International, Roskilde, Denmark). The amount of dried leaves used was calculated based on the annual average amount of fallen leaves per unit area. A total of 10 mL agar (0.5% w/v) (Agar powder, gelling temperature 30-31 °C, Nacalai Tesque, Inc.) was added in two layers (5 mL each) above the dried leaves, in each well. A slight modification was made to the original Sandwich method, any floating dry leaves were sunk to the bottom of dish, to avoid direct contact between the lettuce root and the testing sample, to minimize the thigmomorphogenesis effect.

After gelatinizing the agar, 5-lettuce seeds (*Lactuca sativa* cv. Great Lakes 366), Takii Co. Ltd. were placed on the surface of each agar-containing well of the multidish. Lettuce was selected as a test plant due to easy handling, high rate of germination and fast growth. The multidish plate was then sealed and kept in an incubator (Biotron LH220S, NK System Co. Ltd.) for three days at 25 °C in dark conditions. Afterwards, the germination and the seedling growth (radicle and hypocotyls length) was measured. As control, an identical multidish plate without the plant material was set under the same conditions described above.

RESULTS AND DISCUSSION

Screening of Plants

Growth rates of the radicle and hypocotyl of the lettuce seedlings for all the tested species are shown in Table 2. The 239 donor species dried leaf material had both inhibitory and stimulatory effects on the radicle and hypocotyl of lettuce seedlings. The data followed a normal distribution. The mean and standard deviation of the percentages were calculated. The criteria of †, ††, ††† in 10 mg dry leaves refer to the radicle growth that is lower than the mean value minus 1(σ), 1.5(σ) and 2(σ) and §, §§ in 50 mg dry leaves to the radicle growth lower than mean value minus 1(σ) and 1.5(σ), respectively. The growth rate of lettuce seedling roots treated with 10 mg dry leaves varied from 0 to 108 % compared to the control (100 %). A total of 238 species out of 239 inhibited the growth and only one species proved stimulatory than control.

Seventeen species caused a growth rate lower than 22.7% (Mean-1.5 σ) compared with the control, of which five species belonged to the Fabaceae family, two to Euphorbiaceae and Asteraceae each and one to Amaranthaceae, Annonaceae, Cleomaceae, Lamiaceae, Loganiaceae, Moraceae, Phyllanthaceae and Verbenaceae each.

(i). **Hypocotyl:** The growth rate of lettuce seedling hypocotyls treated with 10 mg dry leaves varied from 0 to 175% (Fig. 2). Of all the species tested, 123 showed hypocotyl growth inhibition and the other 116 species displayed growth promoting activity. Those species causing root growth of less than 13.3% (Mean-2σ), also showed hypocotyl inhibition with a growth rate of less than 36%, proving to be highly inhibitory to lettuce seedling growth. However, those with root lengths of more than 20% had hypocotyl lengths that fluctuated from 37% to 175%.

Table 2. Radicle and hypocotyl growth rate of 239 Paraguayan plants tested by sandwich method.

Family	Scientific Name	Col. Site	Leaf biomass rate (mg) Radicle (10)	Hyp. (10)	Radicle (50)	Hyp. (50)	Criteria※
	Herbaceous Plants						
Acanthaceae	*Ruellia simplex* C.Wright	c	54	129	27	94	
Amaranthaceae	*Alternanthera brasiliana* (L.) Kuntze	a	59	137	25	96	
Amaranthaceae	*Alternanthera pungens* Kunth	a	25	48	13	31	†/§
Amaranthaceae	*Amaranthus hybridus* subsp. *quitensis* (Kunth) Costea & Carretero	c	38	107	21	67	
Amaranthaceae	*Dysphania ambrosioides* (L.) Mosyakin & Clemants	a	32	72	10	24	†/§
Amaranthaceae	*Gomphrena macrocephala* A.St.-Hil.	b	40	78	21	47	
Amaranthaceae	*Gomphrena* sp.	a	21	36	10	19	††/§
Amaranthaceae	*Iresine diffusa* Humb. & Bonpl. ex Willd.	a	58	140	23	84	
Amaranthaceae	*Pfaffia glomerata* (Spreng.) Pedersen	a	33	80	18	43	
Apiaceae	*Eryngium elegans* Cham. & Schltdl.	a	65	117	45	99	
Apiaceae	*Hydrocotyle bonariensis* Comm. ex Lam.	a	70	139	41	116	
Apiaceae	*Hydrocotyle ranunculoides* L.	b	24	54	10	54	†/§
Araceae	*Philodendron bipinnatifidum* Schott ex Endl.	b	45	75	18	54	
Aristolochiaceae	*Aristolochia gibertii* Hook.	a	68	117	41	71	
Asteraceae	*Chaptalia nutans* (L.) Polák	a	25	59	8	38	†/§
Asteraceae	*Acanthospermum australe* (Loefl.) Kuntze	a	82	130	62	115	
Asteraceae	*Acanthospermum hispidum* DC.	a	57	104	45	87	
Asteraceae	*Achyrocline alata* (Kunth) DC.	a	72	126	51	102	
Asteraceae	*Ambrosia artemisiifolia* L.	a	37	105	15	64	
Asteraceae	*Aspilia camporum* Chodat.	b	33	61	26	56	
Asteraceae	*Baccharis trimera* (Less.) DC	a	66	141	39	97	
Asteraceae	*Bidens pilosa* L.	a	33	74	45	82	
Asteraceae	*Calea verticillata* (Klatt) Pruski.	b	72	114	50	100	
Asteraceae	*Campuloclinium macrocephalum* (Less.) DC.	a	18	54	0	0	††/§§
Asteraceae	*Chrysolaena platensis* (Spreng.) H.Rob.	a	63	120	22	71	
Asteraceae	*Eupatorium inulifolium* Kunth	a	44	107	24	86	
Asteraceae	*Isostigma riedelii* Sch.Bip. ex Baker	a	68	86	35	55	
Asteraceae	*Pectis odorata* Griseb.	a	48	80	32	70	
Asteraceae	*Pluchea sagittalis* Less.	a	66	125	41	85	
Asteraceae	*Porophyllum lanceolatum* DC.	a	84	134	34	79	
Asteraceae	*Stevia* sp.	a	19	95	14	55	††/§
Asteraceae	*Stevia entreriensis* Hieron. ex Arechav.	a	39	88	27	71	
Asteraceae	*Stevia rebaudiana* (Bertoni) Bertoni	a	54	120	45	104	
Asteraceae	*Trixis pallida* Less.	a	60	113	38	81	
Asteraceae	*Vernonia oligolepis* Sch.Bip. ex Baker	b	61	89	51	95	
Asteraceae	*Viguiera linearifolia* Chodat	a	43	75	26	54	
Asteraceae	*Xanthium spinosum* L.	a	48	97	26	79	
Bignoniaceae	*Dolichandra unguis-cati* (L.) L.G.Lohmann	c	50	111	28	78	
Caryophyllaceae	*Stellaria media* (L.) Vill.	a	54	108	20	71	
Commelinaceae	*Commelina erecta* L.	a	55	102	27	76	
Convolvulaceae	*Ipomoea bonariensis* Hook.	a	50	123	25	96	
Costaceae	*Costus arabicus* L.	a	65	109	29	78	
Cyperaceae	*Kyllinga vaginata* Lam.	a	57	112	39	110	
Cyperaceae	*Rhynchospora* sp.	b	84	131	46	123	
Cyperaceae	*Scleria latifolia* Sw.	b	85	135	60	124	
Cyperaceae	*Scleria* sp.	a	67	130	49	130	

Table 2. (Cont.)

Family	Scientific Name	Col. Site	Leaf biomass rate Radicle (10mg)	Hyp. (10mg)	Radicle (50mg)	Hyp. (50mg)	Criteria※
Equisetaceae	*Equisetum giganteum* L.	a	92	127	73	129	
Euphorbiaceae	*Croton bonplandianus* Baill.	a	65	101	31	66	
Euphorbiaceae	*Croton hirtus* L'Hér.	a1	40	83	24	63	
Euphorbiaceae	*Croton* sp.	a	38	115	22	71	
Euphorbiaceae	*Euphorbia serpens* Kunth	c	41	98	19	57	
Fabaceae	*Chamaecrista desvauxii* (Collad.) Killip	b	72	93	44	101	
Fabaceae	*Crotalaria incana* L.	a	53	113	16	70	
Fabaceae	*Crotalaria pallida* Aiton	a	40	90	18	68	
Fabaceae	*Crotalaria pumila* Ortega	b	49	101	34	95	
Fabaceae	*Crotalaria stipularia* Desv.	a	44	100	35	92	
Fabaceae	*Desmodium affine* Schltdl.	a	61	108	27	79	
Fabaceae	*Rhynchosia minima* (L.) DC.	a	51	124	22	84	
Fabaceae	*Senna* sp.	a	11	29	6	18	†††/§§
Fabaceae	*Zornia crinita* (Mohlenbr.) Vanni	b	38	82	23	74	
Heliconiaceae	*Heliconia* sp.	b	47	102	28	80	
Iridaceae	*Eleutherine bulbosa* (Mill.) Urb.	a	65	110	39	81	
Iridaceae	*Neomarica* sp.	b	69	116	58	105	
Iridaceae	*Sisyrinchium pachyrhizum* Baker	b	64	126	57	137	
Iridaceae	*Sisyrinchium* sp.	b	54	128	24	77	
Lamiaceae	*Hyptis suaveolens* (L.) Poit.	a	56	86	21	58	
Lamiaceae	*Leonurus sibiricus* L.	a	19	58	6	19	††/§§
Lamiaceae	*Ocimum ovatum* Benth.	a	65	110	33	76	
Lamiaceae	*Scutellaria racemosa* Pers.	a1	44	89	27	84	
Lythraceae	*Cuphea lysimachioides* Cham. & Schltdl.	a	60	122	35	76	
Lythraceae	*Cuphea racemosa* (L.f.) Spreng.	a	57	109	48	97	
Lythraceae	*Heimia salicifolia* (Kunth) Link	c	69	102	36	77	
Malpighiaceae	*Bunchosia armeniaca* (Cav.) DC.	a	60	81	24	61	
Malvaceae	*Cienfuegosia drummondii* (A.Gray) Lewton	a	48	120	30	98	
Malvaceae	*Sida rhombifolia* L.	a	44	100	18	73	
Malvaceae	*Sida* sp.	c	49	135	22	76	
Martyniaceae	*Craniolaria integrifolia* Cham.	a	41	63	14	26	§
Melastomastaceae	*Acisanthera alsinaefolia* (DC.) Triana	b	36	75	10	29	§
Moraceae	*Dorstenia brasiliensis* Lam.	c	42	94	19	77	
Nyctaginaceae	*Boerhavia diffusa* L.	a	29	63	14	41	†/§
Orchidaceae	*Catasetum fimbriatum* (C.Morren) Lindl.	b	40	74	16	50	
Orchidaceae	*Miltonia flavescens* (Lindl.) Lindl.	b	76	123	54	123	
Orchidaceae	*Oeceoclades maculata* (Lindl.) Lindl.	a	81	139	28	94	
Passifloraceae	*Passiflora cincinnata* Mast.	a	37	58	18	41	
Phyllanthaceae	*Phyllanthus niruri* L.	a	22	44	6	25	††/§§
Phytolaccaceae	*Petiveria alliacea* L.	a	30	36	21	29	†
Poaceae	*Andropogon lateralis* Nees	a	74	137	53	135	
Poaceae	*Axonopus pressus* (Steud.) Parodi	b	69	110	51	99	
Poaceae	*Chusquea ramosissima* Lindman	b	43	88	30	83	
Poaceae	*Digitaria insularis* (L.) Mez ex Ekman	a	30	70	16	55	†
Poaceae	*Elionurus muticus* (Spreng.) Kuntze	a	61	86	28	67	
Poaceae	*Elionurus* sp.	a	59	103	15	39	
Poaceae	*Eragrostis polytricha* Nees	b	48	95	28	79	
Poaceae	*Melinis repens* (Willd.) Zizka	a	27	77	18	68	†
Poaceae	*Paspalum distichum* L.	a	52	87	28	62	
Poaceae	*Pharus lappulaceus* Aubl.	a	27	56	13	40	†/§
Poaceae	*Sporobolus junceus* (P.Beauv.) Kunth	a	48	79	25	63	

Table 2 (Cont.)

Family	Scientific Name	Col. Site	Leaf biomass rate Radicle (10mg)	Hyp. (10mg)	Radicle (50mg)	Hyp. (50mg)	Criteria※
Polygonaceae	*Persicaria punctata* (Elliott) Small	a	58	117	49	102	
Polypodiaceae	*Microgramma persicariifolia* (Schrad.) C. Presl	b	39	85	27	77	
Portulacaceae	*Portulaca grandiflora* Hook.	a	28	90	12	54	†/§
Pteridaceae	*Adiantopsis radiata* (L.) Fée	b	48	106	45	99	
Pteridaceae	*Adiantum orbignyanum* Kuhn	a	73	108	38	94	
Pteridaceae	*Adiantum serratodentatum* Humb. & Bonpl. ex Willd.	b	76	103	58	102	
Pteridaceae	*Cheilanthes microphylla* (Sw.) Sw.	a	58	108	46	98	
Rubiaceae	*Richardia brasiliensis* Gomes	a	47	108	21	86	
Rubiaceae	*Spermacoce verticillata* L.	b	62	117	30	98	
Schizaeaceae	*Anemia phyllitidis* (L.) Sw.	b	66	105	29	69	
Schizaeaceae	*Anemia tomentosa* (Savigny) Sw.	b	26	58	0	0	†/§§
Schizaeaceae	*Lygodium volubile* Sw.	b	92	121	68	66	
Scrophulariaceae	*Scoparia dulcis* L.	a	37	66	18	44	
Solanaceae	*Physalis viscosa* L.	a1	34	112	10	71	§
Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	a	63	156	32	97	
Typhaceae	*Typha domingensis* Pers.	c	26	68	11	47	†/§
Verbenaceae	*Aloysia polystachya* (Griseb.) Moldenke	a	47	109	11	8	§
Verbenaceae	*Glandularia megapotamica* (Spreng.) Cabrera & G.Dawson	a	29	67	13	49	†/§
Verbenaceae	*Stachytarpheta cayennensis* (Rich.) Vahl	a	63	104	39	76	
Verbenaceae	*Verbena litoralis* Kunth	a	86	152	52	120	
Zingiberaceae	*Hedychium coronarium* J.Koenig	a	61	98	28	71	
	Woody Plants						
Anacardiaceae	*Anacardium humile* A.St.-Hil.	b	41	130	30	120	
Acanthaceae	*Justicia brasiliana* Roth	b	53	118	31	89	
Anacardiaceae	*Lithraea molleoides* (Vell.) Engl.	a	58	96	26	76	
Anacardiaceae	*Schinopsis quebracho-colorado* (Schltdl.) F.A. Barkley & T. Mey.	c	74	99	45	88	
Anacardiaceae	*Schinus molle* L.	a	85	135	62	147	
Annonaceae	*Annona cacans Warm.*	a	47	84	32	66	
Annonaceae	*Annona crotonifolia* Mart.	b	53	74	24	66	
Annonaceae	*Annona dioica* A. St.-Hil.	a	64	116	36	122	
Annonaceae	*Duguetia furfuracea* (A.St.-Hil.) Saff.	b	36	69	28	78	
Annonaceae	*Rollinia emarginata* Schltdl.	a	10	36	3	22	†††/§§
Apocynaceae	*Aspidosperma australe* Müll. Arg.	a	72	135	47	95	
Apocynaceae	*Aspidosperma quebracho-blanco* Schltdl.	c	69	116	61	110	
Aquifoliaceae	*Ilex paraguariensis* A. St. Hil.	b	66	130	42	115	
Arecaceae	*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.	a	64	106	41	99	
Arecaceae	*Allagoptera leucocalyx* (Drude) Kuntze	b	70	102	59	102	
Arecaceae	*Butia campicola* (Barb.Rodr.) Noblick	b	73	111	58	116	
Arecaceae	*Butia paraguayensis* (Barb.Rodr.) L.H.Bailey	b	80	136	74	145	
Arecaceae	*Copernicia alba* Morong.	a	84	138	78	134	
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman	b	74	103	56	117	
Arecaceae	*Trithrinax schizophylla* Drude	c	37	88	23	80	
Asclepiadaceae	*Funastrum bonariense* (Hook. & Arn.)Schltr.	c	39	74	22	73	
Asparagaceae	*Cordyline congesta* (Sweet) Steud.	b	59	80	59	97	
Asparagaceae	*Herreria montevidensis* Klotzsch ex Griseb.	a	37	64	16	40	
Asteraceae	*Cyclolepis genistoides* D. Don.	c	75	118	38	109	
Asteraceae	*Dasyphyllum brasiliense var. divaricatum* (Griseb.) Cabrera	a	80	136	43	114	
Asteraceae	*Jungia floribunda* Less.	a	69	101	36	66	
Asteraceae	*Pluchea dodonaeifolia* (Hook. & Arn.) H.Rob. & Cuatrec.	a	42	62	26	45	

Table 2 (Cont.)

Family	Scientific Name	Col. Site	Leaf biomass rate Radicle (10mg)	Hyp. (10mg)	Radicle (50mg)	Hyp. (50mg)	Criteria ※
Bignoniaceae	*Handroanthus impetiginosus* (Mart. ex DC.) Mattos	a	59	114	32	104	
Bignoniaceae	*Jacaranda cuspidifolia* Mart.	a	68	129	54	117	
Bignoniaceae	*Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S.Moore	b	69	85	34	57	
Bignoniaceae	*Tabebuia nodosa* (Griseb.) Griseb.	c	61	124	43	108	
Bombacaceae	*Ceiba insignis* (Kunth) P.E.Gibbs & Semir	c	50	132	31	106	
Bombacaceae	*Ceiba speciosa* (A.St.-Hil.) Ravenna	c	74	105	25	71	
Boraginaceae	*Cordia americana* (L.) Gottschling & J.S.Mill.	c	70	145	40	99	
Boraginaceae	*Cordia ecalyculata* Vell.	a	69	174	52	153	
Buddlejaceae	*Buddleja madagascariensis* Lam.	a	73	130	30	84	
Capparaceae	*Anisocapparis speciosa* (Griseb.) Cornejo & Iltis	c	45	72	28	49	
Capparaceae	*Cappariсordis tweedieana* (Eichler) Iltis & Cornejo	c	26	75	13	53	†/§
Capparaceae	*Capparis salicifolia* Griseb.	c	78	99	43	64	
Caricaceae	*Jacaratia spinosa* (Aubl.) A.DC.	b	58	110	25	81	
Caryocaraceae	*Caryocar brasiliense* A.St.-Hil.	b	23	43	10	22	†/§
Celastraceae	*Maytenus ilicifolia* Mart. ex Reissek	b	26	70	8	48	†/§
Cleomaceae	*Cleome aculeata L.*	a	0	0	0	0	†††/§§
Convolvulaceae	*Cochlospermum regium* (Schrank) Pilg.	b	39	85	27	79	
Convolvulaceae	*Ipomoea carnea* Jacq.	b	33	70	17	49	
Erythroxylacea e	*Erythroxylum cuneifolium* (Mart.) O.E.Schulz	b	38	89	23	82	
Euphorbiaceae	*Acalypha communis* Müll.Arg.	a	67	148	39	145	
Euphorbiaceae	*Croton solanaceus* (Müll.Arg.) G.L.Webster	b	50	86	37	80	
Euphorbiaceae	*Croton urucurana* Baill.	b	54	93	45	85	
Euphorbiaceae	*Jatropha isabellei* Müll.Arg.	a	61	111	45	108	
Euphorbiaceae	*Microstachys hispida* (Mart.) Govaerts	b	11	35	6	17	†††/§§
Euphorbiaceae	*Stillingia scutellifera* D.J.Rogers	b	15	59	7	30	††/§
Fabaceae	*Acacia aroma* Hook. & Arn.	c	36	74	24	63	
Fabaceae	*Acacia farnesiana* (L.) Willd.	a	48	88	22	62	
Fabaceae	*Acacia* sp.	c	51	94	33	65	
Fabaceae	*Anadenanthera colubrina* (Vell.) Brenan	b	22	55	8	28	††/§
Fabaceae	*Bauhinia rufa* (Bong.) Steudel.	b	47	81	36	78	
Fabaceae	*Caesalpinia paraguariensis* (Parodi) Burkart	c	44	75	17	54	
Fabaceae	*Inga semialata* (Vell.) C.Mart.	b	42	71	30	68	
Fabaceae	*Mimosa dolens* Vell.	b	50	104	37	83	
Fabaceae	*Myrocarpus frondosus* Allemão.	a	53	110	28	70	
Fabaceae	*Parapiptadenia rigida* (Benth.) Brenan	a	60	122	42	123	
Fabaceae	*Prosopis alba* Griseb.	c	48	70	23	51	
Fabaceae	*Prosopis kuntzei* Kuntze	c	55	131	44	109	
Fabaceae	*Prosopis nigra* Hieron.	c	81	173	43	134	
Fabaceae	*Prosopis ruscifolia* Griseb.	c	11	30	8	23	†††/§
Fabaceae	*Pterogyne nitens* Tul.	c	6	0	3	0	†††/§§
Fabaceae	*Senna alata* (L.) Roxb.	a	38	62	15	49	
Fabaceae	*Senna occidentalis* (L.) Link	c	17	66	8	37	††/§
Fabaceae	*Senna pendula* (Willd.) H.S.Irwin & Barneby	c	59	115	46	101	
Lamiaceae	*Minthostachys* sp.	a	43	51	0	0	§§
Lauraceae	*Nectandra angustifolia* (Schrad.) Nees & Mart.	a	44	74	14	41	§
Loganiaceae	*Strychnos brasiliensis* (Spreng.) Mart.	c	4	7	3	5	†††/§§
Malpighiaceae	*Heteropterys glabra* Hook. & Arn.	a	49	98	23	99	
Malvaceae	*Gossypium barbadense* L.	a	41	93	29	84	
Meliaceae	*Cabralea canjerana* (Vell.) Mart.	b	35	104	22	103	
Meliaceae	*Cedrela fissilis* Vell.	b	43	79	26	66	

Table 2 (Cont.)

Family	Scientific Name	Col. Site	Leaf biomass rate Radicle (10mg)	Hyp. (10mg)	Radicle (50mg)	Hyp. (50mg)	Criteria ※
Moraceae	*Ficus enormis* (Miq.) Miq.	a	65	113	50	125	
Moraceae	*Maclura tinctoria* (L.) D.Don ex Steud.	a	63	122	30	99	
Moraceae	*Morus alba* L.	a	58	99	30	85	
Moraceae	*Sorocea bonplandii* (Baill.) W.C.Burger, Lanj. & de Boer	b	6	20	6	12	†††/§§
Myrtaceae	*Campomanesia adamantium* (Cambess.) O.Berg	b	78	86	45	91	
Myrtaceae	*Campomanesia xanthocarpa* (Mart.) O.Berg	a	77	175	42	122	
Myrtaceae	*Myrceugenia* sp.	a	108	88	50	50	
Myrtaceae	*Myrcia guianensis* (Aubl.) DC.	b	57	109	37	83	
Myrtaceae	*Psidium australe* var. *argenteum* (O.Berg) Landrum	b	34	60	22	47	
Myrtaceae	*Psidium guajava* L.	a1	45	106	31	63	
Myrtaceae	*Psidium guineense* Sw.	a	67	111	60	105	
Nyctaginaceae	*Pisonia aculeata* L.	a	29	95	26	83	†
Piperaceae	*Piper regnellii* (Miq.) C.DC.	b	48	61	13	29	§
Polygonaceae	*Salta triflora* (Griseb.) Adr. Sanchez	c	37	86	32	91	
Primulaceae	*Myrsine parvula* (Mez) Otegui	b	52	104	26	57	
Rhamnaceae	*Ziziphus mistol* Griseb.	c	69	142	46	122	
Rosaceae	*Rubus imperialis* Cham. & Schltdl.	a	60	102	23	67	
Rubiaceae	*Calycophyllum multiflorum* Griseb.	c	71	89	48	62	
Rubiaceae	*Coussarea platyphylla* Müll.Arg.	b	69	133	44	118	
Rutaceae	*Esenbeckia densiflora* (Chodat & Hassl.) Hass.	a	32	49	9	10	§
Rutaceae	*Helietta apiculata* Benth.	a	50	103	23	45	
Rutaceae	*Zanthoxylum fagara* (L.) Sarg.	a	72	97	23	50	
Rutaceae	*Zanthoxylum petiolare* A.St.-Hil. & Tul.	a	79	131	41	118	
Salicaceae	*Salix humboldtiana* Willd.	c	59	93	24	52	
Sapindaceae	*Serjania erecta* Radlk.	b	38	90	26	80	
Smilacaceae	*Smilax goyazana* A.DC.	b	37	56	18	35	
Solanaceae	*Solanum granuloso-leprosum* Dunal	b	34	89	24	77	
Solanaceae	*Solanum lycocarpum* A. St.-Hil.	b	52	97	14	36	
Solanaceae	*Solanum paniculatum* L.	a	41	99	21	75	
Solanaceae	*Solanum sisymbriifolium* Lam.	a	36	66	4	11	§§
Theophrastaceae	*Clavija nutans* (Vell.) B.Ståhl	b	61	117	33	79	
Tiliaceae	*Luehea candicans* Mart.	b	69	86	51	97	
Ulmaceae	*Celtis tala* Gillies ex Planch.	c	42	156	28	124	
Urticaceae	*Cecropia pachystachya* Trécul	b	52	98	43	96	
Urticaceae	*Cecropia peltata* L.	b	60	144	26	94	
Verbenaceae	*Aloysia citriodora* Palau	a	43	51	0	0	§§
Verbenaceae	*Aloysia gratissima* (Gillies & Hook.) Tronc.	a	63	81	37	65	
Verbenaceae	*Aloysia virgata* (Ruiz & Pav.) Juss.	a	44	118	18	81	
Verbenaceae	*Lantana* sp.	a	33	85	22	72	
Verbenaceae	*Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson	a	52	135	35	107	
Verbenaceae	*Lippia lycioides (Cham.) Steud.*	a	15	6	10	5	††/§
Verbenaceae	*Priva boliviana* Moldenke	c	42	98	23	63	
Vochysiaceae	*Vochysia tucanorum* Mart.	b	58	95	34	98	
Zygophyllaceae	*Bulnesia sarmientoi* Lorentz ex Griseb.	c	27	58	17	49	†
	Mean		51.0	96.3	29.7	74.7	
	SD (σ)		18.9	30.8	15.6	32.5	
	Mean-σ		32.1	65.6	14.1	42.2	
	Mean-1.5σ		22.7	50.2	6.3	25.9	
	Mean-2σ		13.3	34.8	-1.5	9.7	

a: Botanical Garden and Zoo of Asunción, a1: Capital and its surroundings, b: Mbaracayú Natural Reserve, c: Chaco Region

※: ††† = Mean-2σ, †† = Mean-1.5σ, and † = Mean-σ, when tested with 10mg dry leaves for radicle
§§ = Mean-1.5σ, and § = Mean-σ, when tested with 50mg dry leaves for radicle

(ii). **Roots:** The species causing high levels of inhibitory activity in roots (Mean-2σ) are marked by a circle on Fig. 2. Within this group are *Cleome aculeata* (Cleomaceae), *Strychnos brasiliensis* (Loganiaceae), *Pterogyne nitens* (Fabaceae), *Sorocea bonplandii* (Moraceae), *Rollinia emarginata* (Annonaceae), *Microstachys hispida* (Euphorbiaceae), *Prosopis ruscifolia* (Fabaceae) and *Senna* sp. (Fabaceae). Using 50 mg dry leaves, the length of lettuce seedling roots varied between 0 and 78%, all of them resulted in inhibition compared to the control (Fig. 3). A total of 214 species showed a growth rate of lettuce seedling roots of less than 50% and only 25 species produced a growth rate of more than 50%. Hypocotyl lengths ranged widely from 0 to 158%, with 190 species causing hypocotyls below 100% and 49 species above 100%. With 50 mg dry leaves, *Anemia tomentosa* (Schizaeaceae), *Aloysia citriodora* (Verbenaceae), *Minthostachys* sp. (Lamiaceae), *Campuloclinium macrocephalum* (Asteraceae) and *C. aculeata* (Cleomaceae) showed a strong allelopathic activity, completely inhibiting the germination of lettuce seeds. A further nine species showed strong growth inhibitory activity of lettuce seedling roots when tested with 50 mg dry leaves, with growth of less than 6.3% (Mean-1.5σ). Relatively strong activity was displayed by 26 species, with a growth rate of less than 14.1% (Mean-σ).

Comparing the allelopathic activity of the samples collected in the three different locations, there were no significant differences between the means among collection sites (Table 3).

In this research, all the species displaying the strongest allelopathic activity using 10 mg dry leaves, belonged to woody species. These plants tend to exhibit stronger activity than herbaceous plants.

From the total 239 samples collected, 178 are known to be medicinal plants (11,34,32,49). The frequency distribution of medicinal plants and non-medicinal plants showed a slight tendency of the former having stronger activity than the latter, revealing similar results to the work by Fujii *et al*. (6) on the allelopathic activity of medicinal plants (Figure not shown).

Growth inhibition was observed more frequently in roots than in hypocotyls. This may be due to the fact that the roots emerge first and therefore they are subject to the effects of allelochemicals leached from the dry leaves, directly and for a longer time.

Characteristics of specific plants

One species that showed strong allelopathic activity is *Cleome aculeata* (Cleomaceae) [syn. *Hemiscola aculeata* (13)]. This is an annual weedy species, 30-50 cm plant height, leaves with stipular spines (46). It is commonly distributed along roadsides, vacant lots, orchards, fields with annual and perennial crops and around crops fields (26,46). It is locally known by the name of "Aña Ka'a" which means "devil's herb" in the Paraguayan native language, Guarani. Several other species of this family, such as *Cleome spinosa*, *C. hassleriana* and *C. gynandra*, are known to release methyl isothiocyanate (MITC), due to the myrosinase hydrolization of glucosinolate content in the plant (8,30,50), which is recognized for its strong biocidal activity (18,28).

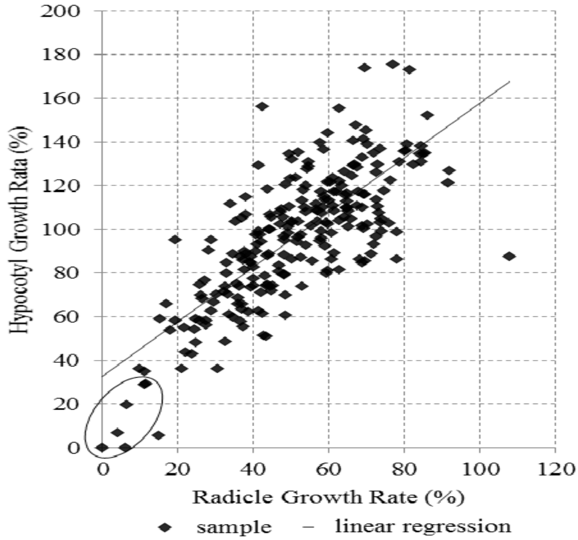

Figure 2. Elongation of lettuce radicle and hypocotyl when tested with 10 mg of dry leaves of the sample plants
Note: Samples in the circle indicate high levels of inhibitory activity on the roots (Mean-2σ)

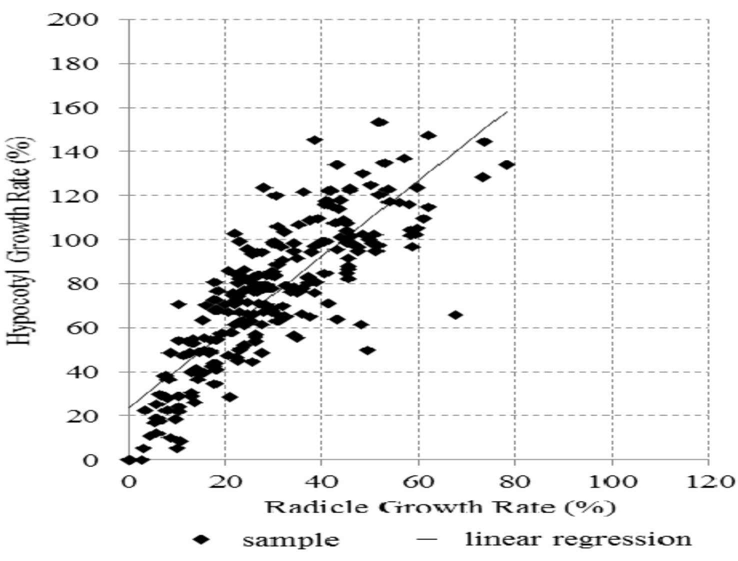

Figure 3. Elongation of lettuce radicle and hypocotyl when tested with 50 mg of dry leaves of the sample plants

Table 3. Mean and standard deviation of tested plants when classified by types of plant and collection site

Classification	n	Root (10 mg)	Hypocotyl (10 mg)	Root (50 mg)	Hypocotyl (50 mg)
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Plant Type					
Medicinal	178	50±19	95±31	29±16	73±33
Non Medicinal	61	53±19	101±29	31±15	79±31
Life Form					
Herbaceous	118	51±18	99±27	29±15	74±29
Woody	121	51±20	94±34	30±16	76±36
Collection site					
Asunción	130	52±19	99±31	29±15	73±34
Mbaracayu	71	51±18	92±26	33±17	78±31
Chaco	38	48±20	96±37	28±14	73±31

No statistical differences among all the categories.

Allelopathic activities of some *Cleome* species have already been reported. Ventura *et al*. (48) found that volatile oil extracted from *Cleome guianensis* inhibit germination and preliminary growth of *Senna occidentalis* but did not identify the allelochemical responsible of the inhibition. Ladhari *et al*. (17), reported the phytotoxic activity of *C. arabica* and isolated the compound 11 -α-acetylbrachy-carpone- 22(23)-ene as the main active allelochemical in this plant.

Jana and Biswas (15) isolated lactam nonanic acid (LNA) from root exudates of *C. viscosa* L. and reported its allelopathic and antimicrobial properties. There is no report of the presence of these compounds in *C. aculeata.*

Strychnos brasiliensis **:** Lettuce seedling roots under the effects of *Strychnos brasiliensis* (Loganiaceae) were strongly inhibited, growing 4% compared to the control (100%), with the hypocotyl growing only 7% under treatment with 10 mg dry leaves. This plant is commonly known in Paraguay as "Ñuati Kurusu" and "Quina Cruzeiro" in Brazil, its bark is used for medicinal purposes, as a febrifuge (11,40). Members of the Loganiaceae family are known to contain alkaloids such as strychnine and brucine, renowned for their high toxicity (31,33). Alkaloids are known to display allelopathic activity; Macías *et al*. (21) presents strychnine as an alkaloid with allelopathic activity.

Pterogyne nitens **:** Roots of lettuce seedlings treated with 10 mg dry leaves of *Pterogyne nitens* (Fabaceae) revealed growth of just 6% compared to the control and the hypocotyl did not emerge at all, showing that this plant has strong allelopathic activity. *P. nitens* is known as "Yvyraro" in Paraguay, "Amendoim" in Brazil and "Tipa Colorado" in Argentina. The wood of this tree is used to make fine furniture and cabinets and it is one of the most common species of the dominant stratum of Eastern Paraguay (Región Oriental). For local medicinal use, the bark of this plant is applied to infected wounds and used to wash ulcers

(11,20). Several research works have been carried out to elucidate the medicinal use of *P. nitens* alkaloids and flavonoids (2,4,45), but there is no research on its allelopathic activity.

Sorocea bonplandii **:** *Sorocea bonplandii* (Moraceae) is commonly known as "Ñandypa'i" and is used as a forage (44). Pharmacological analysis of this plant showed analgesic and antiulcerogenic effects (9). In Paraguay, it is used in folk medicine for weight loss and to reduce cholesterol level (49). *S. bonplandii* is a dominant species of the sub-canopy stratus of the sub-tropical Atlantic forest, exhibiting a characteristic clumped spatial distribution. This dominance is also seen in the eco-region of Selva Tropical in Eastern Paraguay (16,20,41,42,). López *et al*. (20) mentioned the negative effect of this plant on the normal growth of other plant seedlings. They attributed this effect to light competition, but had not considered other possibilities such as allelopathy.

Maraschin-Silva and Aqüila (23) assessed the allelopathic potential of aqueous leaf extracts of five Brazilian species, including *Sorocea bonplandii*. They observed that the extracts of the tested plants delayed lettuce germination and exerted a negative effect on seedling growth, but they didn't elucidate the allelochemicals present in each plant. In our research, this plant also showed a strong allelopathic activity toward lettuce seedlings, inhibiting 94% of root growth and 80% of hypocotyl growth, when treated with 10 mg dry leaves.

Rollinia emarginata **:** *Rollinia emarginata* (Annonaceae) also exhibited strong allelopathic activity; the growth rate of the roots of lettuce seedlings was 10% and hypocotyl length 36% compared to the control, when tested with 10 mg dry leaves. *R. emarginata* is a typical species of the intermediate stratum of Paraguayan forest. It is commonly known in Paraguay by "Aratiku", the Guarani name for "Cherimoya". The fruits are edible and both the leaves and fruits are used in folk medicine (11,20). Fujii *et al*. (6) reported strong inhibitory activity of *Annona cherimola*, a species closely related to *R. emarginata*. They also found other members of the Annonaceae with strong allelopathic activity.
Microstachys hispida (Euphorbiaceae) (syn. *Sebastiania hispida*) is an herb or subshrub, commonly known in Brazil as "Mercúrio" (35). Some researchers have studied its medicinal and acaricidal activities (39,43), but not its allelopathic activity.

Others : Leaf infusion of *Prosopis ruscifolia* (Fabaceae), known as "Viñal" in Paraguay, is used by the locals to treat conjunctivitis (10). Nowadays the flour of this plant seed is used to prepare gluten-free bread (1).
Morikawa *et al* (27) tested 170 Peruvian plants for their allelopathic activity against lettuce radicle growth using the same sandwich method (10 mg dry leaves between two agar layers). Four species [*Bidens pilosa*, (Asteraceae), *Psidium guajava* (Myrtaceae), *Lippia alba* (Verbenaceae) and *Verbena litoralis* (Verbenaceae)] were in common, but same as this work, none of them showed a remarkable allelopathic activity.

None of the above-mentioned species, except *Sorocea bonplandii*, has been reported previously as for allelopathic potential. This study is the first large scale screening of

Paraguayan plants for their allelopathic activity, giving the results of several plant species with high allelopathic potential.

Further investigation on the allelochemicals and field test of these plants may lead to the use of extracts or residues of these plants as natural herbicides, therefore reducing the use of synthetic agrochemicals, which may help to alleviate environmental deterioration. Incorporating allelopathy into agricultural management such as cover crop or mulching may be an alternative practice for sustainable agricultural development.

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