ELSEVIER

Journal of Ethnopharmacology 45 (1995) 35–41

Journal of ETHNO-PHARMACOLOGY

Mutagenicity, insecticidal and trypanocidal activity of some Paraguayan Asteraceae

A. Rojas de Arias*[a], E. Ferro[a,b], A. Inchausti[a], M. Ascurra[a], N. Acosta[a], E. Rodriguez[a], A. Fournet[c]

[a]*Instituto de Investigaciones en Ciencias de la Salud (I.I.C.S.), Rio de la Plata y La Gerenza, C.P. 2511, Paraguay*
[b]*Facultad de Ciencias Quimicas (UNA), C.P. 1055, Asunción, Paraguay*
[c]*Institut Français de Recherches Scientifique pour le Développement en Coopération (O.R.S.T.O.M.), C.P. 97, Asunción, Paraguay*

Received 14 September 1994; revision received 14 September 1994; accepted 19 September 1994

Abstract

The insecticidal, moulting inhibition and trypanocidal effects of crude extracts of 7 Paraguayan Asteraceae were evaluated on *Triatoma infestans* and bloodstream forms of *Trypanosoma cruzi*, respectively. Both mutagenicity and toxicity were evaluated by sister chromatid exchange (SCE) in human peripheral lymphocyte culture and by the lethality test of *Artemia salina*. The ethanolic extracts from *Chromolaena christieana* (stem and bark), *Achyrocline satureoides* (leaves and flowers) and *Mikania cordifolia* (root and stem), at a concentration of 250 μg/ml, showed the highest percentage of lysis on bloodstream forms of *Trypanosoma cruzi*. The extracts of *Chromolaena christieana* and *Achyrocline satureoides* also presented high mutagenic and toxic capacity when they were evaluated by the SCEs assay and *Artemia salina* test, respectively. Insecticidal activity was only observed in the hexane extract of flowers of *Achyrocline satureoides* (45% of mortality), when 0.05 μg of crude concentration was applied on *Triatoma infestans*. The ethanolic extracts of stem from *Mikania cordifolia* and *Vernonia brasiliana* inhibited the moulting of *Triatoma infestans* when it was compared with their controls. Since no ethnobotanical information on these plants has been found related to similar use in Paraguay, our findings suggest, for the first time, the potential anti-trypanocidal and moulting inhibition of these Asteraceae.

Keywords: Asteraceae; *Trypanosoma cruzi*; Trypomastigote forms; *Triatoma infestans*; Insecticide; Paraguay

1. Introduction

Chagas' disease is a major health problem in Latin America. Prevalence varies from 5–60% and 16–18 million people are infected, with a further 90 million at risk (WHO, 1991). Chagas' disease is caused by *Trypanosoma cruzi*, a protozoan parasite which is mainly transmitted by insect vectors (subfamily Triatominae).

Lack of vaccine or suitable drugs means Chagas' disease control relies on control of vector achieved through insecticide application. However, reinfestation of sprayed houses can be a major problem caused by lack of sufficient residual effect of insecticides (Schofield and Dias, 1991). Lack of control in blood banks and the disadvantages

* Corresponding author.

0378-8741/95/$09.50
SSDI 0378-8741(94)01193-4

caused by the gentian violet are also contributing to the transmission of Chagas' disease by blood transfusion (Littlefield et al., 1985; Rezende et al., 1965).

The effect of plant-derived and synthetic compounds on *Trypanosoma cruzi* has been reported by several authors (Pinto et al., 1987; Moncada et al., 1989), especially from the Asteraceae, such as *Senecio* sp. (Sarti et al., 1984), *Vernonia* sp., *Eupatorium* sp. (Cardozo and Lopez, 1987) and *Munnozia maronii* (André) H. Robinson (Fournet et al., 1993). Recently Chiari et al. (1991) have tested a number of compounds where a sesquiterpene lactone and 3 compounds having a benzoquinone moiety presented high activity against the bloodstream forms of *Trypanosoma cruzi*.

Efforts to control the vector have been carried out with the oils and plant extracts of the seeds of Meliaceae (Schumutterer, 1988). Leite et al. (1987) have reported the insecticidal effect of *Ruta graveolens* L. (Rutaceae) and *Eucalyptus argenteum* L. (Myrtaceae) on nymphs of *Dipetalogaster maximus* (Triatominae). Arias and Schmeda-Hirschmann (1988) have observed the repellent effect of the oil and ethanolic extract of *Melia azedarach* L. (Meliaceae) fruits on nymphs of *Triatoma infestans*. The ethanolic extract of leaves of *Tagetes minuta* L. (Asteraceae) have shown an interesting insecticidal activity on nymphs of *Rhodnius neglectus* (Triatominae); *Tagetes erecta* L. extract has also exhibited a moulting inhibitory effect (Schmeda and Rojas de Arias, 1992).

Although plants are used by Paraguayan populations as acaricides, insecticides or as therapeutic agents (Correia, 1984) very little has been published about their insecticidal or trypanocidal activities. The family Asteraceae has been widely used as medicinal plants in Paraguay and their antiparasitic properties are well known (Gonzalez Torres, 1981). Some Asteraceae like *Chrysanthenum* sp. (Zito et al., 1983) are recognized as insecticides and their commercial exploitation has been described (Bezanger-Beauquesne, 1969). In the present work, the trypanocidal effect of crude extracts of some Paraguayan Asteraceae on the bloodstream forms of *Trypanosoma cruzi*, as well as their insecticidal and moulting inhibition effect on *Triatoma infestans*, were assessed. The active extracts were also evaluated for mutagenicity by the test of induction of sister chromatid exchange (SCE) in human peripheral lymphocyte culture.

2. Materials and methods

2.1. Plant material

Plants were collected and identified by I. Basualdo and N. Soria in the Departments of Concepción, Central and Presidente Hayes of Paraguay between February and December 1991. Voucher herbarium specimens have been deposited at the Herbarium of the Department of Botany (FCQ) of the Faculty of Chemical Sciences (UNA), Asunción, Paraguay. Crude extracts were prepared from 7 plants belonging to the family Asteraceae. The samples of the plants were dried, pulverized and subjected to exhaustive successive extraction with hexane and 95% ethanol.

2.2. Biological assays

2.2.1. Parasites. Albino mice infected with *Trypanosoma cruzi* Y strain, 7 days after infection were used. Blood was obtained by cardiac puncture using 3.8% sodium citrate as anticoagulant in a 7:3 blood/anticoagulant ratio. The parasitemia in infected mice ranged from 1×10^5–5×10^5 parasites/ml. Plants extracts were dissolved in cold dimethyl sulfoxide (DMSO) to a final concentration of 250 μl/ml. Aliquots of 10 μl of each extract of different concentrations (4, 20, 40, 100 and 250 μg/ml) were mixed in microtiter plates with 100 μl of infected blood containing different parasite concentrations (1×10^5 and 10^6 parasites/ml). Infected blood and infected blood containing gentian violet at 250 μg/ml were used as controls. The plates were shaken for 10 min at room temperature and kept at 4°C for 24 h. Each solution was microscopically observed at 400 ×, placing a 5 μl sample on a slide and covering it with a 22 × 22 mm coverglass for parasite counting (Schempler, 1978).

2.2.2. Insects. For the extract application on Triatominae insects 50 μg of the extract were topically applied to each insect. Hexane extracts were dissolved in ethanol or acetone while the ethanolic extracts were dissolved in 95% ethanol.

Stock solutions containing 50 mg/ml of extract were prepared for each sample and 1 μl aliquots of such solution were applied on the insect abdominal tergites.

Triatoma infestans fourth-stage nymphs were bred in laboratory conditions using a technique previously described (Schmeda and Rojas de Arias, 1992). They were maintained at 28°C and 60–70% humidity. They were fed on pigeons for 20 min, 6 h later individual insects were treated topically with an aliquot of one of the test extracts. Control insects were topically treated with solvents. After 25 days nymphal mortality and moulting inhibition were recorded and the Cochran test was used to test the significant difference between the treatments and the controls (Sokal and James, 1984).

2.2.3. Induction of sister chromatid exchange (SCE). Human lymphocytes were obtained from healthy donors by vein puncture and cultures were prepared using Hungerford's modified method (Rossner et al., 1987). One half ml of heparinized blood was incubated in 5 ml of RPMI 1640 medium supplemented with 10% bovine serum, 0.1 ml phytohemagglutinin and 7.5% sodium bicarbonate at 37°C for 72 h. Tested materials were dissolved in DMSO and added to the culture. 5-Bromodeoxyuridine (BrDU) was added to each culture to a final concentration of 5 μg/ml. Incubation was performed in the dark to avoid SCE induced by photolysis of BrDU-substituted DNA. After 2 rounds of replication, demecolcine (0.05 μg/ml) was added to each culture 2 h before harvesting of cells. Cells were treated with an hypotonic solution, fixed with methanol/acetic acid and dropped on clean chilled glass slides. Differential sister chromatids staining was performed as described by Perry and Wolff (1974). In each sample, 20 metaphases showing differences in chromatid staining were observed and photographed. Terminal SCE was scored as 2. SCE in the centromeric region was not scored because they were indistinguishable from the twisting of sister chromatids. Negative controls with DMSO (1%) were included to establish spontaneous frequency of aberrant cells. The median test was used to test the significant difference between the treatments and controls (Sokal and James, 1994).

2.2.4. Artemia salina bioassay. The concentration of each crude extract that caused 50% mortality (LD_{50}) of brine shrimp larvae was calculated using the methodology described by Meyer et al. (1982).

3. Results and discussion

In the present study 7 Paraguayan Asteraceae were evaluated. Table 1 shows that the ethanolic crude extracts from *Chromoleana christieana* (stem and root), *Achyrocline satureioides* (leaves and flowers) and *Mikania cordifolia* (root and leaves) present at the highest percentage of lysis on bloodstream forms of *Trypanosoma cruzi*. We have observed that hexane extracts produce lower lysis than ethanolic extracts. In earlier phytochemical studies, sesquiterpene lactones had been isolated from *Chromoleana glaberrima* (Ahmed et al., 1986), *Mikania micrantha* (Becker et al., 1987) and *Mikania urticifolia* (Gutteriez et al., 1988) or diterpenes from *Mikania alvimii* (Bohlmann et al., 1982), from ethanolic extracts. Trypanocidal activities in *Chromoleana christieana* (ex *Eupatorium*) extracts have been previously reported (Cardozo et al., 1988). Carvalho et al. 1991 have published the antiparasitic activity of *Vernonia brasiliana* extracts against *Plasmodium berghei*, but we had found low levels of activity in the different extracts (hexane and ethanolic leaves) of this plant against *Trypanosoma cruzi*.

In 1990, Gonzalez et al. evaluated several extract and pure compounds from Chilean Asteraceae against *Trypanosoma cruzi* trypomastigotes. A *Baccharis boliviensis* (Wedd.) Cabr. extract showed a total trypomastigote lysis at 4°C for 24 h at 500 μg/ml. Better results were found in our study, with *Baccharis notosergila* Griseb. which showed a high activity percentage against *Trypanosoma cruzi* trypomastigotes (69.4%) at the same conditions but at lower concentration (250 μg/ml). In a recent study, we have published the in vitro activity of crude Asteraceae extracts towards trypomastigotes forms of *Trypanosoma cruzi* (Rojas de Arias et al., 1994). In our experience, a limited number of tested extracts have shown an important activity against *Trypanosoma cruzi*. Nevertheless, natural products isolated from plant

Table 1
Activity of some Paraguayan Asteraceae against the vector of Chagas' disease (*Triatoma infestans*), the in vitro bloodstream forms of *Trypanosoma cruzi* and toxicity against *Artemia salina*

Plant species (vouchers)	Vernacular name[a]	Therapeutic uses	Plant part used[b]	Extract/principle	Mortality of *Triatoma infestans* (%)	Moulting inhibition of *Triatoma infestans* (%)	Activity against *Trypanosoma cruzi* at 250 μg/ml (%)	Toxicity on *Artemia salina* LC_{50} (μg/ml)
				Ivermectin	0	0		
				Gentian violet			100	
Achyrocline satureioides (Lam.) DC. (N. Soria 5040)	Marcela, marcelita (S), yataí ca'a (G)	Digestive, cholagogue, antidiabetis	St, Lf	Hexane	8.3	10	41.2	66.8
			Fl	Hexane	45*	35	61.6	100
			Lf, Fl	Ethanol	0	30	78.0	>1000
			Fl	Ethanol			79.4	>1000
Baccharis notosergila Griseb. (N. Soria 4584)	carqueja (S)	Antirheumatic	St	Ethanol	15	15	69.4	>1000
			Rt	Ethanol	10	15	61.3	57.8
Chromoleana christieana (Baker) K. et R. (N. Soria 4585)	Typychá pito (G)	Aromatic	Rt	Ethanol	8.3	25	91.9	>1000
			St	Ethanol	0	2.5	88.9	>1000
			Lf	Ethanol	8.3	57.4*	0	>1000
Gochnatia barrosii Cabrera (N. Soria 4526)			Fl, Lf	Hexane	15	47.3*	0	>1000
			Fl, Lf	Ethanol	0	8.3	0	>1000
			St	Ethanol	0	20	0	>1000
Gochnatia polymorpha (Less.) Cabrera (N. Soria 4491)	Cambará (G), tatané-moroti (G), curalotodo (S)	Expectorant, cough, anti-asthmatic	Lf	Hexane	0	5	0	>1000
			Lf	Ethanol	15	29.4	54.5	>1000
			St	Ethanol	0	60.0*	0	>1000
Mikania cordifolia (L. f) Willd. (N. Soria 4586)	Guaco (S)	Anti-snakebites	St	Ethanol	0	73.3*	11.4	>1000
			Rt, Lf	Ethanol	8.3	25	87.1	>1000
Vernonia brasiliana (L.) Duke (R. Degen 2052)			St	Ethanol	0	66.7*	0	>1000
			Lf	Hexane	0	16	30.7	>1000
			Lf	Ethanol	5	25	27.3	>1000

[a]G, guarani; S, Spanish.
[b]Fl, flowers; Lf, leaves; Rt, roots; St, stem.
*Statistically significant $P < 0.001$ (Cochran test) when compared with their controls.

Table 2
Frequency of sister chromatid exchanges (SCE) in peripheral lymphocytes induced by plant extracts at different concentrations

Botanical name	Plant part used[a]	Extract/ principle	Concentrations (μg/ml)						
			5	10	25	50	75	100	DMSO (1%)
		Ivermectin				4.2 ± 0.8	7.7 ± 1.4*	8.8 ± 2.3*	4.2 ± 0.8
		Benznidazole	3.3 ± 0.8	4.2 ± 1.2	7.7 ± 0.9*	12.6 ± 2.2*			3.4 ± 0.9
		Cyclophos-phamide			10.3 ± 2.0	12.8 ± 2.9*	18.9 ± 3.2*	23.8 ± 3.6*	9.2 ± 1.9
Chromolaena christieana (Baker) K. et R.	Rt	Ethanol			16.3 ± 1.9*	21.3 ± 3.2*	22.3 ± 3.2*	26.8 ± 4.6*	9.1 ± 1.9
	St	Ethanol			12.4 ± 2.8*	18.2 ± 3.0*	20.9 ± 2.4*	21.7 ± 3.2*	8.9 ± 2.3
Gochnatia barrosii Cabrera	Fl, Lf	Hexane			5.4 ± 0.9	8.1 ± 1.4*	18.9 ± 2.6*	22.0 ± 2.2*	5.8 ± 1.3
Gochnatia polymorpha (Less.) Cabrera	Lf	Hexane			11.1 ± 2.3	16.3 ± 3.4*	17.9 ± 3.8*	24.8 ± 3.7*	10.0 ± 2.0
	Fl, Lf	Ethanol			4.6 ± 0.9	6.6 ± 0.9*	13.2 ± 2.0*	20.2 ± 3.2*	4.8 ± 1.2
Vernonia brasiliana (L.) Duke	St	Ethanol			6.2 ± 0.9	10.0 ± 2.1*	15.0 ± 2.2*	17.5 ± 2.6*	5.9 ± 0.9

Values are presented as mean ± S.D.
[a]Fl, flowers; Lf, leaves; Rt, roots; St, stem.
*Statistically significant to $P < 0.001$ (median test).

extracts have shown high trypanocidal activity and can provide alternatives to replace toxic synthetic drugs.

Concerning insecticidal activity only the hexane extract of flowers of *Achyrocline satureoides* showed an insecticidal activity against *Triatoma infestans* (45% of mortality), but this extract did not present any moulting inhibition effect (Table 1). Ethanolic extracts of stems of *Mikania cordifolia* and *Vernonia brasiliana* were the most active extracts to inhibit the moulting of *Triatoma infestans*, such results were statistically significant when they were compared with their controls. As stated earlier, some of these plants contains sesquiterpene lactones and diterpenes (Herz and Kulanthaivel, 1985; Alarcon et al., 1990), some of which are known to inhibit insects moulting and have been recommended as biorational compounds for use in insect control programmes (Bellés, 1988).

As for the mutagenic capacity evaluated by SCE's assay (see Table 2), *Chromoleana christieana* extracts were found to be extremely mutagenic in all tested concentrations; these effects were statistically significant when concentrations were higher than 10 μg/ml (Table 2). The extracts of *Gochnatia polymorpha* and *Vernonia brasiliana* also showed a high mutagenic capacity.

No ethnobotanical information on these plants has been found relating to insecticidal or parasiticidal popular use in Paraguay. However, a relevant relationship of insecticidal and trypanocidal effects of hexane extracts of *Achyrocline satureoides* and its toxic activity against *Artemia salina* has been observed. The trypanocidal activity of the ethanolic extracts of *Chromoleana christieana* and its mutagenic power have been also noticed in this study.

Acknowledgements

This work was supported by a Grant for Scientific Research from Deutsche Gesellschaft für Technische Zusammernarbeit (GTZ). Thanks are due to Dr R. Kiefer, expert from GTZ, for the interest and encouragement. We also thank Prof. I. Basualdo, N. Soria and N. Alvarenga for the plant collection and extract preparation.

References

Ahmed, A.A., Whittemore, A.T. and Mabry, T.J. (1986) A guaianolide from *Chromoleana glaberrima*. *Journal of Natural Products* 49, 363–364.

Alarcon, M.C.B., Lopes, J.L.C. and Herz, W. (1990) Glaucolide B, a molluscicidal sesquiterpene lactone and other constituents of *Vernonia eremophila*. *Planta Medica* 56, 271–273.

Arias, A.R. and Schmeda-Hirschmann, G. (1988) The effects of *Melia azederach* on *Triatoma infestans* bugs. *Fitoterapia* 59, 148–149.

Bézanger-Beauquesne, L. (1970) Chrysanthèmes insecticides. *Plantes Medicinales et Phythoterapie* 3, 269–309.

Becker, R., Japupovic, J., Bohlmann, F. and Schmeda-Hirschmann, G. (1987) Germacra-1,10 *Z*, 4 *E*-dien-12, 8a-olides from *Mikania micrantha*. *Planta Medica* 53, 105–106.

Bellés, X. (1988) *Insecticidas Biorracionales*. Consejo Superior de Investigaciones Cientificas, Colección Nueva Tendencias 9, p. 405, Madrid, España.

Bohlmann, F., Adler, A., King, R.M. and Robinson, H. (1982) Ent-labdanes from *Mikania alvimii*. *Phytochemistry* 21, 173–176.

Cardozo, J.E. and Lopez, J.L. (1987) Actividade in vitro dos estratos das plantas, *Vernonia* sp. e *Eupatorium* sp. contra formas sanguineas da cepa Y do *Trypanosoma cruzi*. *Memorias do Instituto Oswaldo Cruz*, Rio de Janeiro, Suppl. 82.

Carvalho, L.H., Brandao, M.G.L., Santos-Filho, D., Lopes, J. L.C. and Kretli, A.U. (1991) Antimalarial activity of crude extracts from Brazilian plants studied in vivo in *Plasmodium berghei* infected mice and in vitro against *Plasmodium falciparum* in culture. *Brazilian Journal of Medical and Biological Research*, 24, 1113–1123.

Chiari, E., Braga de Oliveira, A., Soares Raslan, D., Mesquita, A.L. and Tavares, K.G. (1991) Screening in vitro of natural products against blood forms of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85, 372–374.

Correia, P. (1984) *Dicionario da Plantas uteis do Brazil e das Exoticas Cultivadas*. Impresa Nacional, IBDF Rio de Janeiro 6, pp. 229–248.

Fournet, A., Muñoz, V., Roblot, F., Hocquemiller, R., Cavé, A. and Gantier, J.C. (1993) Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from *Munnozia maronii* (Asteraceae). *Phytotherapy Research* 7, 111–115.

Goncalves, A., Vasconcellos, M., Docampo, R., Cruz, F., Souza, W. and Leon, W. (1980) Evaluation of the toxicity of 3-allyl-α-lapachone against *Trypanosoma cruzi* bloodstream forms. *Molecular and Biochemical Parasitology* 1, 167–176.

Gonzalez, J.H., Sagua, J., Araya, A., Loyola, G., Morales, J., Pereira, J. and Estrada, M. (1990) In vitro activity of natural products against the trypomastigotes forms of *Trypanosoma cruzi*. *Phytotherapy Research*, 4, 1–4.

Gonzalez Torres, D.M. (1981) *Catalogo de plantas medicinales*

usadas en Paraguay. Asunción, Paraguay, Ed. Comuneros, pp. 1–456.

Guttierez, A.B., Oberti, J.C. and Herz, W. (1988) Germacran-5,14,6,12-diolides from *Mikania urticifolia*. *Phytochemistry* 27, 938–939.

Herz, W. and Kulanthaivel, P. (1985) Diterpenes and sesquiterpene lactones from *Mikania congesta*. *Phytochemistry* 24, 1761–1768.

Leite, F.E.M., Zapata, M.T.G., Soares, V.A. and Marsden, P. D. (1987) Avaliacao laboratorial de insecticidas de origen vegetal utilizando *Dipetalogaster maximus* como agente de teste. *Memorias Instituto do Oswaldo Cruz*, Rio de Janeiro Suppl. II 84, 122.

Littlefield, N.A., Blackwell, B.N., Hewitt, D. and Gaylor, D. (1985) Chronic toxicity and carcinogenicity studies of Gentian Violet in Mice. *Fundamental Applied Toxicology* 5, 902–912.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. (1982) Brine shrimp: a convenient general bioassay for active plants constituents. *Planta Medica* 45, 31–34.

Moncada, C., Repetto, Y., Aldunate, J., Letellier, M.E. and Morello, A. (1989) Role of glutathione in the susceptibility of *Trypanosoma cruzi* to drugs. *Comparative Biochemistry and Physiology*, Part C 94, 87–91.

Perry, P. and Wolff, S. (1974) New Giemsa method for the differential staining of sister chromatid. *Nature* 251, 156–158.

Pinto, A.V., Ferreira, V.F., Capella, R.S., Gilbert, B., Pinto, M.C.R. and Da Silva, J.S. (1987) Activity of some naphthoquinones on blood stream forms of *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81, 609–610.

Rezende, J.M., Zupelli, W. and Bequetto, M.G. (1965) O problema da transmissao da doença de Chagas por transfusao de sangre. *Revista Goiana de Medicina* 11, 35–47.

Rojas de Arias, A., Inchausti, A., Ascurra, M., Fleitas, N., Rodriguez, E. and Fournet, A. (1994) In vitro activity and mutagenicity of bisbenzylisoquinolines and quinones against *Trypanosoma cruzi* trypomastigotes. *Phytotherapy Research*, 8, 141–144.

Rossner, P., Cerna, M., Lepsi, P. and Pohlova, H. (1987) Mutagenicity of some substituted 1-phenyl-3,3-dimethyltrazene. II. Chromosomal aberrations in human peripheral lymphocytes in vitro. *Mutation Research* 190, 183–183.

Sarti, S.J., Silva, D., Santos Filho, D., Petto, G., Ferreira, S., Rossi, M. and Dos Santos, R. (1984) Controle de transmissao transfusional da doença de Chagas avaliacao de drogas isoladas a partir de productos naturais vegatais. *XI Reuniao Annual en Pesquiça Basica en Doença de Chagas* - Caxambù, M.G., Brasil, abstracts P 146, 20–22.

Schlemper, B.R. (1978) Estudos experimentais de quimioprofilaxia da transmissao de doença de Chagas por transfusao sanguinea. *Revista de Patologia Tropical* 7, 55–111.

Schmeda-Hirschmann, G. and Rojas de Arias, A. (1992) A screening method for natural products on triatomine bugs. *Phytotherapy Research* 6, 68–73.

Schofield, C.J. and Dias, J.C.P. (1991) A cost benefit analysis of Chagas' disease control. *Memorias do Instituto Oswaldo Cruz* 86, 285–295.

Schumutterer, H. (1986) Insect growth disrupting and fecundity, reducing ingredients from the neem and China Berry. In: D.H. Morgan and B.N. Mandeva (Eds.), *CRC Handbook of Natural Pesticides*, vol. 3. Insect Growth regulations, Part. B. CRC Press, Boca Raton, pp. 119–170.

Sokal, R.R. and James, F. (1984) Biometria. *Principios y Metodos Estadisticos en la Investigación Biologià*. Blume H. Ed., Barcelona, España.

WHO/TDR. (1991) *Control of Chagas' Disease*. WHO Technical Report Series 811, Geneva, pp. 95.

Zito, S.W., Zieg, R.G. and Staba, E.J. (1983) Distribution of pyrethrins in oil glands and leaf tissue of *Chrysanthenum cineriaefolium*. *Planta Medica* 47, 205–207.