Screening of Asteraceae (Compositae) Plant Extracts for Larvicidal Activity against *Aedes fluviatilis* (Diptera: Culicidae)

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Ethanol extracts of 83 plants species belonging to the Asteraceae (Compositae) family, collected in the State of Minas Gerais, Brazil, were tested for larvicidal activity against the mosquito Aedes fluviatilis *- Diptera: Culicidae). The extract from* Tagetes minuta *was the most active with a LC_{90} of 1.5 mg/l and LC_{50} of 1.0 mg/l. This plant has been the object of several studies by other groups and its active components have already been identified as thiophene derivatives, a class of compounds present in many Asteraceae species. The extract of* Eclipta paniculata *was also significantly active, with a LC_{90} of 17.2 mg/l and LC_{50} of 3.3 mg/l and no previous studies on its larvicidal activity or chemical composition could be found in the literature. Extracts of* Achryrocline satureoides, Gnaphalium spicatum, Senecio brasiliensis, Trixis vauthieri, Tagetes patula *and* Vernonia ammophila *were less active, killing more than 50% of the larvae only at the higher dose tested (100 mg/l).*

Key words: mosquitoes - larvicidal - *Aedes fluviatilis* - Asteraceae - plant extracts

The selective pressure of conventional insecticides is enhancing resistance of mosquito populations at an alarming rate (Brown 1986), increasing the demand for new products that are environmentally safe, target-specific and degradable.

Co-evolution has equipped plants with a plethora of chemical defenses against insect predators. Aware of this effect, mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Balandrin 1985, Rawls 1986, Sukamar et al. 1991). Natural insecticides such as pyrethrum, rotenone and nicotine, among others, have been extensively used until recently for insect control (Balandrin 1985). Limonoids such as azadirachtin and gedunin, present in species from the Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world (Dua et al. 1995, Nagpal et al. 1996). Recently, the discovery of insecticide activity of phototoxins present in Asteraceae species has stimulated the interest in this plant family as part of the search for new plant derived insecticides (Rawls 1986).

In Brazil, the resurgence of several mosquito transmitted diseases such as malaria, dengue and yellow fever, together with the appearance of insect resistance to conventional insecticides, stresses the necessity for the search for new insecticides. Aiming for the discovery of cost effective alternatives for the control of disease vector insects, we decided to evaluate the toxicity of crude ethanol extracts of 83 Asteraceae species from our local flora against the larvae of *Aedes (Ochlerotatus) fluviatilis* (Lutz, 1904). This mosquito shares many biological characteristics with *Ae. aegypti*, the vector of yellow fever, and has been shown to be an useful model in biological studies of experimental infections and insecticide susceptibility tests (Consoli & Williams 1978, 1981, Camargo et al. 1983).

MATERIALS AND METHODS

Plant collection - The plants (Table I) were collected in Belo Horizonte and its vicinities, in the State of Minas Gerais, Brazil. After botanical

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TABLE I

Asteraceae plant species collected for testing against 4th instar larvae of *Aedes (Ochlerotatus) fluviatilis* (Lutz, 1904)

Entry	Plants	Common name	Habitat[a]	BHCB[b]
1.	*Acanthospermum australe* (Loef.) Kunt.	carrapicho de carneiro	W	19056
2.	*Achyrocline alata* DC.	macela	S	19017
3.	*Achyrocline satureiodes* (Lam.) DC.	macela do campo	S	19067
4.	*Actinoseris angustifolia* (Gard) Cabr.	-	S	19086
5.	*Ageratum conyzoides* L.	catinga de bode	C	19031
6.	*Alomia myriadenia* Baker	-	S	19013
7.	*Aspilia jolyana* G.M. Barroso	-	S	19200
8.	*Aspilia serrulata* Baker	-	S	19019
9.	*Baccharis dracunculifolia* DC.	-	S	19048
10.	*Baccharis helichrysoides* DC.	-	C	19101
11.	*Baccharis platypoda* DC.	-	S	19063
12.	*Baccharis serrulata* (Lam.)Pers.	suncho	S	19054
13.	*Baccharis trimera* (L.) DC.	carqueja	S	19077
14.	*Baccharis trinervis* Pers.	-	W	19062
15.	*Bidens gardneri* Baker	-	C	19028
16.	*Bidens pilosa* L.	picão	S	19014
17.	*Bidens rubifolius* HBK	-	C	19023
18.	*Blainsvillea biaristata* DC.	-	W	19052
19.	*Centratherum punctatum* Cass.	-	W	19045
20.	*Chaptalia nutans* (L.) Polak	fumo do mato	W	19089
21.	*Cosmus sulphureus* Cav.	picão grande	W	19046
22.	*Dasyphyllum macrocephala* Baker	-	S	19076
23.	*Eclipta paniculata* Hassk.	-	W	19037
24.	*Elephantopus mollis* HBK	erva colégio	C	19032
25.	*Emilia sonchifolia* DC.	pincel de estudante	W	19034
26.	*Eremanthus glomerulatus* (DC.) Less	-	S	19073
27.	*Eremanthus sphaerocephalus* (DC.) Baker	chapéu de couro	S	19058
28.	*Erigeron bonariensis* L.	erva lanceta	W	19090
29.	*Erigeron canadensis* L.	-	W	19039
30.	*Eupatorium amphidictyum* DC.	-	S	19065
31.	*Eupatorium bupleurifolium* DC.	guaco	S	19051
32.	*Eupatorium aff. capilare* DC.	-	C	19029
33.	*Eupatorium halimifolium* DC.	-	S	19102
34.	*Eupatorium klenioides* HKB	-	C	19024
35.	*Eupatorium laevigatum* Lam.	cambaré	S	19050
36.	*Eupatorium squalidum* DC	erva São João	C	19022
37.	*Galinsoga parviflora* Cav.	picão branco	S	19015
38.	*Gnaphalium spicatum* Hook	macela	W	19088
39.	*Gochnatia polymorpha* (Less.) Cabr.	cambará do mato	S	19078
40.	*Hypochaeris brasiliensis* Griseb	chicória do campo	W	19092
41.	*Jaegeria hirta* (Lag.) Less	jeguéria	W	19040
42.	*Jungia floribunda* Less	arnica	W	19057
43.	*Lychnophora pinaster*	-	S	19020
44.	*Lychnophora pseudovillosissima* Semir & L.F.	-	S	19103
45.	*Mikania aff. premnifolia* Gardn.	-	S	19096
46.	*Mikania cordifolia* (L.F.) Wild	guaco	S	19047
47.	*Mikania lutzelburghii* Mattf.	-	S	19061
48.	*Mikania obtusata* DC.	-	S	19069
49.	*Mikania sessilifolia* DC.	cundurango	S	19060
50.	*Piptocarpa rotundifolia* Baker	paratudo	C	19087
51.	*Pluchea quitoc* L.	quitoco	W	19036
52.	*Porophillum ruderale* (Jacq.) Cass.	couve cravinho	C	19030
53.	*Pseudoginonoxis pohlii* (Sch.-Bip.) L.F.	-	S	19098
54.	*Pterocaulon alopecureoides* (Lam.) DC.	barbasco	C	19025
55.	*Senecio brasiliensis* Less	flor das almas	C	16377
56.	*Senecio confusus* Britten	-	W	19035
57.	*Siegesbeckia orientalis* L.	-	W	19091

Entry	Plants	Common name	Habitat[a]	BHCB[b]
58.	*Sonchus oleraceus* L.	serralha lisa	W	19038
59.	*Spilanthes paniculata* Well ex. DC.	-	W	19196
60.	*Stenocline chionaea* DC.	-	S	19064
61.	*Symphyopappus polystachyus* Baker		C	19059
62.	*Symphyopappus reticulatus* Baker	-	S	19100
63.	*Tagetes minuta* L.	coarí bravo	W	19055
64.	*Tagetes patula* L.	cravo de defunto	W	00373
65.	*Taraxacum officinale* (With.) Wiggers	dente de leão	W	19070
66.	*Tithonia rotundifolia* Baker	-	W	19026
67.	*Trixis vauthieri* DC.	celidônia	S	19072
68.	*Vanillosmopsis erithropappa* Sch.-Bip.	cambará	S	19082
69.	*Verbesina clausseni* Sch.-Bip.	cravo do campo	S	19016
70.	*Vernonia ammophila* Gardn.	-	C	19027
71.	*Vernonia condensata* Baker	-	W	19081
72.	*Vernonia crotonoides* (DC.) Sch.-Bip.	cambará	S	19021
73.	*Vernonia ferruginea* Less.	assa-peixe do Pará	C	19083
74.	*Vernonia grandiflora* Less.	saudades do campo	W	19195
75.	*Vernonia herbaceae* (Vell.) Rusby	-	W	19041
76.	*Vernonia linearis* Spreng	-	S	19066
77.	*Vernonia pedunculata* DC.	-	S	19084
78.	*Vernonia polyanthes* (Spreng.) Less	cambará guaçu	W	19080
79.	*Vernonia remotiflora* Rich.	-	W	19053
80.	*Vernonia varroniaefolia* DC.	-	W	19042
81.	*Viguiera ovatifolia* Baker	-	S	19074
82.	*Wedelia paludosa* DC.	margaridão	W	19033
83.	*Wulfia baccata* (L.F.) Kuntz	-	W	19093

a: C: Cerrado (savanna); S: stony areas; W: weeds. *b:* voucher specimen code at BHCB Herbarium, Departamento de Botânica, Universidade Federal de Minas Gerais, Brasil.

identification, voucher specimens were deposited in the BHCB Herbarium at the Department of Botany, Federal University of Minas Gerais.

Extracts preparation - The aerial parts of the plants were dried in the shade, ground in a knife mill or in a homogenizer and extracted twice (24 hr) with ethanol (95%) at room temperature. The solvent was removed by rotary evaporation under reduced pressure at temperature below 45°C. The resulting crude extracts were stored in a freezer at -20°C until assayed. Immediately before running the bioassay, sufficient amounts of extract were transferred to a vial and the residual solvent removed under high vacuum for at least 24 hr.

Bioassay - Stock solutions of each extract were prepared at 1000 mg/l by sonicating them in a ultrasound bath (45 kHz, 100W) for 5-10 min. Test solutions of 100, 10 and 1 mg/l were then prepared by diluting the stock solution in tap water. The extracts were tested against young fourth instar *Ae.(Ochlerotatus) fluviatilis* (Lutz, 1904) larvae from a colony maintained at the Centro de Pesquisas René Rachou (Consoli & Lourenço-de-Oliveira 1994). Each dilution was placed in sterile glass dishes (9 cm diam./150 ml capacity) and 30 larvae were added. After 24 hr contact at room temperature, the number of dead larvae in each dish was counted. The larvae were considered dead if they were immobile and unable to reach the water surface. Previous experiments showed no significant differences in mortality when the assay was extended to 48 hr (Consoli et al. 1988). The ambient temperature during all experiments ranged between 23-28°C. Control experiments without extract were run in parallel and the mortality was always bellow 4.5%. All experiments were run in triplicate.

Statistical evaluation - Mortality means were compared using Duncan's Test (Edwards 1960) at the alpha significance level of 0.05; LC_{50} and LC_{90} were calculated for the most active extracts using probit analysis (Armitage & Berry 1987).

RESULTS AND DISCUSSION

Eighty-three species, belonging to 48 genera of the Asteraceae family were collected for this survey. Table I lists all plants in alphabetical order and includes their habitats, BHCB herbarium codes and common name when available (Corrêa 1984). The genera *Baccharis*, *Eupatorium*, *Mikania* and *Vernonia* were the best represented, with at least five species each.

Table II summarizes the results of the bioassays for those species that promoted statistically significant mortality, using Duncan's significance test (Edwards 1960), for at least one concentration when compared to the control. Larvicidal activities higher than 50% at any tested concentration were highlighted.

The crude extract from the aerial parts of *T. minuta* (Table II, entry 22) displayed an LC_{90} and an LC_{50} of 1.5 and 1.0 mg/l, respectively, making it the most active of all extracts tested. 5-*E*-ocimenone was initially described as the active component of *Tagetes minuta* (Maradufu et al. 1978) but Green et al. (1991) suggested that further compounds, not identified by them, were also responsible for the observed toxicity towards mosquito larvae. More recently, four thiophene derivatives were identified from a larvicidal floral extract fraction of this plant (Perich et al. 1995). This fraction displayed an LC_{50} of 3.9 against *Ae. aegypti* and *Anopheles stephensi* 3rd instar larvae, i. e., four times less potent than the crude extract tested here.

T. patula extract (Table II, entry 23) which is also known to contain thiophene derivatives (Bicchi et al. 1992) was, on the other hand, much less active than *T. minuta*, a result that is in agreement with other published works (Green et al. 1991, Wells et al. 1993, Perich et al. 1994).

The extract of *Eclipta paniculata* was the second most active of the 83 tested in this screening. It promoted the death of 83% of the larvae at 10 mg/l and presented LC_{90} and LC_{50} values of 17.2 and 3.3 mg/l, respectively. No studies describing its insecticide activity or chemical fractionation has been found in the literature. However, considering the chemistry of the genus *Eclipta* (Singh 1988), it is conceivable that thiophene and polyacetylene derivatives are also present in *E. paniculata* and could account for its larvicidal properties. A bioassay-guided fractionation of *E. paniculata* extract will be necessary to confirm this hypothesis.

The extracts of *Achryrocline satureoides*,

TABLE II

Extracts that caused significant lethality of *Aedes fluviatilis* fourth instar larvae [a]

Entry	Plants	% mortality		
		concentration (mg/l)		
		100	10	1
1.	*Achyrocline alata*	14.4	2.2	1.1
2.	***Achyrocline satureoides***	**52.2**	4.4	5.6
3.	*Ageratum conyzoides*	11.1	1.1	2.2
4.	*Alomia myriadenia*	20.0	4.4	1.1
5.	*Aspilia serrulata*	12.2	14.2	2.2
6.	*Bidens pilosa*	12.2	2.2	-
7.	*Chaptalia nutans*	34.4	-	2.2
8.	***Eclipta paniculata***	**98.8**	**83.3**	17.8
9.	*Emilia sonchifolia*	44.4	18.9	12.2
10.	*Eremanthus glomerulatus*	13.3	7.8	6.7
11.	*Erigeron bonariensis*	34.4	2.2	-
12.	*Eupatorium aff. capilare*	11.1	5.6	2.2
13.	***Gnaphalium spicatum***	**76.7**	2.2	1.1
14.	*Jaegeria hirta*	32.2	4.4	6.7
15.	*Jungia floribunda*	32.2	-	-
16.	*Lychnophora pinaster*	48.9	1.1	2.2
17.	*Mikania sessifolia*	25.6	3.3	-
18.	*Pluchea quitoc*	40.0	1.1	1.1
19.	*Porophillum ruderale*	13.3	5.6	1.1
20.	*Pterocaulon alopecureoides*	10.0	3.3	2.2
21.	***Senecio brasiliensis***	**54.4**	2.2	-
22.	***Tagetes minuta***	**100.0**	**100.0**	**48.9**
23.	***Tagetes patula***	**65.6**	25.6	23.3
24.	*Tithonia rotundifolia*	20.0	8.9	-
25.	***Trixis vauthieri***	**56.6**	13.3	2.2
26.	*Verbesina clausseni*	26.7	5.6	2.2
27.	***Vernonia ammophila***	**93.3**	2.2	1.1

a: experiments run in triplicate, using 90 larvae for each concentration. The mortality in the control without extract was always bellow 4.4%

Gnaphalium spicatum, *Senecio brasiliensis*, *Trixis vauthieri* and *Vernonia ammophila* were much less active than those discussed above. Concentrations of 100 mg/l for each extract were necessary to kill more than 50% of the larvae (Table II). The extract of *V. ammophila*, for example, showed LC_{90} and LC_{50} values of 87.8 and 40 mg/l, respectively. To the best of our knowledge this is the first time the larvicidal activity of these species has been described. The extract of *T. vauthieri* has already been the object of phytochemical studies (Bohlmann et al. 1981, Ribeiro et al. 1994) and has been shown to contain 7-methoxyaromadendrin, a larvicidal flavonoid (Echeverry et al. 1992) that could account for its activity. In addition to these studies, a bioassay-guided chemical fractionation protocol should be conducted in order to identify further larvicidal components in this extract.

Except for the extracts discussed above, all others listed in Table II were unable to kill more than 50% of the larvae at the highest concentration tested (100 mg/l) and were considered weakly active. Concerning these species, comparison of our results to those found in the literature yielded the following observations: a) the extract of *Ageratum conyzoides*, reported to be larvicidal in a previous work (Sujatha et al. 1988), was devoid of activity in the present trial; b) the genera *Bidens*, *Mikania* and *Verbesina*, known to contain species with pronounced insecticide activity (Heal et al. 1950, Consoli et al. l988), afforded no larvicidal extract under our experimental conditions; c) extracts of *A. australe*, *A. conyzoides*, *B. pilosa*, *E. bonariensis*, *J. floribunda*, *P. ruderale*, *P. alupecuroides* and *V. claussenii* showed no effect over *Ae. fluviatilis* larvae in this study but have, according to previous works (Macêdo 1995), interfered with oviposition behavior in this species suggesting that different components in the extracts are responsible for these effects; d) while Heal et al. (1950) described the activity of five *Baccharis* species against *Ae. aegypti* and *An. quadrimaculatus* larvae in his survey (Heal et al. 1950), none of the six *Baccharis* species tested here were larvicidal. These inconsistencies in activities may be attributable to seasonal fluctuations in the biosynthesis of the active components, differences in extraction methods, bioassay protocols or difficulties in species authentication (Farnsworth 1966).

In conclusion, from this screening several larvicidal extracts were detected among local Asteraceae species, some of them already described by other research groups. *T. minuta* was the most active and thiophene derivatives were identified as its larvicidal components (Perich et al. 1995). The larvicidal flavonoid 7-metho-xyaromadendrin is present in *T. vauthieri* extracts (Bohlmann et al. 1981, Ribeiro et al. 1994) and could account, at least in part, for its larvicidal activity. Finally, the extract of *E. paniculata* showed strong activity and, as it has not yet been subjected to any phytochemical investigation, it is a good candidate for a bioassay-guided fractionation to identify its larvicidal constituents. It is conceivable that its active components are also thiophene or polyacetylene derivatives, compounds very common in the genus *Eclipta*. Studies to confirm this hypothesis are underway.

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