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An Application of the Brine Shrimp Bioassay for General Screening of Brazilian Medicinal Plants

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SUMMARY. Extracts of eleven species of Brazilian flora were subjected to a bioscreening study to detect cytotoxic activity by the brine shrimp lethality bioassay. The plants studied were: *Baccharis pseudotenuifolia, Baccharis ligustrina, Baccharis platypoda, Baccharis coridifolia, Polygala paniculata, Polygala sabulosa, Croton celtidifolius, Cyathea phalerata, Trichilia catigua, Eugenia uniflora* and *Schinus molle.* The results obtained for the crude extracts of *B. pseudotenuifolia, B. ligustrina, B. coridifolia, P. sabulosa,* and *B. platypoda* ($CHCl_3$ extract), and *C. celtidifolius* (ethanol leaf extract) were promising. These results suggest that more specific bioassays should be encouraged on those plant extracts in order to confirm these conclusions.

RESUMEN. "Aplicación del bioensayo de *Artemia salina* en el análisis general de plantas medicinales brasileñas". Extractos de once especies de la flora brasileña fueron estudiados para evaluar la actividad citotóxica por el test de *Artemia salina.* Las plantas seleccionadas fueron: *Baccharis pseudotenuifolia, Baccharis ligustrina, Baccharis platypoda, Baccharis coridifolia, Polygala paniculata, Polygala sabulosa, Croton celtidifolius, Cyathea phalerata, Trichilia catigua, Eugenia uniflora* y *Schinus molle.* Los resultados obtenidos para los extractos crudos de *B. pseudotenuifolia, B. ligustrina, B. coridifolia, P. sabulosa, B. platypoda* ($CHCl_3$ extract) y el extracto etanólico de *C. celtidifolius* (hojas) fueron promisorios. Estudios más específicos deben ser realizados sobre las plantas que mostraron actividad para este bioensayo para confirmar estas conclusiones.

INTRODUCTION

Over the last decade, interest in drugs of plant origin has been growing steadily. The study of bioactive compounds from plant sources and extracts in the chemical laboratory is often hampered by the lack of a suitable, simple, and rapid screening procedure. There are, of course, many procedures for bioassay, but unless collaborative programs with biologists or pharmacologists are in place, the typical chemical laboratory is not suitably equipped to perform the usual bioassays with whole animals or isolated tissues and organs, as well aseptic techniques [1].

When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds [2].

The *in vivo* lethality in a simple zoological organism, such as the brine shrimp lethality test (BST), developed for Meyer *et al.* [3], might be used as a simple tool to guide screening and fractionation of physiologically active plant extracts, where one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive.

This general bioassay detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply pharmacology at a higher dose, thus if we find toxic compounds, a lower, non-toxic, dose might elicit a useful, pharmaco-

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logical, perturbation on a physiologic system [4]. However, it has been demonstrated that BST correlates reasonably well with cytotoxic and other biological properties [4]. Brine shrimp have been previously utilized in various bioassay systems. There have been many reports on the use of this animal for environmental studies [5-7], screening for natural toxins [8,9] and as a general screening for bioactive substances in plant extracts [3].

Brazilian plants have a long history of use in traditional medicine and even today a large proportion of the population relies solely on the administration of plant-derived preparations for the treatment of a diversity of ailments. Considering that a major challenge today is the discovery of plants with promising activities and the isolation of active principles, we have applied in this work the brine shrimp test (BST) for general activity screening of several extracts of plants from the Mata Atlântica, widely used by people to treat several diseases.

MATERIALS & METHODS

Plant material

The plant species analyzed (Table 1) representing seven families of the Brazilian flora commonly used in traditional medicine, were collected and identified by comparison with authenticated specimens and the voucher of each species was deposited in the herbarium of the Departamento de Botânica da Universidade Federal do Paraná, Curitiba. *Baccharis pseudotenuifolia* was collected around Porto Alegre, Rio Grande do Sul, identified by Dr. Nelson Matzembacher and a voucher is deposited in Instituto de Biociência da Universidade Federal do Rio Grande do Sul. *B. ligustrina* and *B. platypoda* were collected in the vicinities of Ouro Preto, Minas Gerais, identified by Dr. José Badini and a voucher is deposited in the herbarium of the Departamento de Botânica, Universidade Federal de Ouro Preto.

Preparation of the plant hydroalcoholic extract

Air-dried samples (150 g) of each plant species were powdered and macerated at room temperature for 15 days in an alcohol/water mixture (4:1, v/v). After filtration, the solvent was removed by rotatory evaporation under reduced pressure and at temperatures below 55 °C.

Plant Species	Family	Trivial name	Part used	Popular use*
Baccharis coridifolia	Asteraceae	Carqueja	leaves	Hepatoprotective agent and Antiinflammatory
Baccharis pseudotenuifolia	Asteraceae	Carqueja	leaves	Hepatoprotective agent and Gastric diseases
Baccharis ligustrina	Asteraceae	Carqueja	leaves	Hepatoprotective agent and Gastric diseases
Baccharis platypoda	Asteraceae	Carqueja	leaves	Hepatoprotective agent and Gastric diseases
Polygala paniculata	Polygalaceae	Timutu	leaves	Antiinflammatory
Polygala sabulosa	Polygalaceae	Timutu	leaves	Antiinflammatory
Croton celtidifolius	Euphorbiaceae	Sangue de adave	bark/leaves	Treatments of wounds
Cyathea phalerata	Cyatheaceae	Xaxim	wood/leaves	Expectorant and kidney diseases
Trichilia catigua	Meliaceae	Catuaba	bark	Tonic, stimulant
Eugenia uniflora	Myrtaceae	Pitanga	leaves	Anti-diabetic
Schinus molle	Anacardiaceae	Aroeira mansa	leaves	Diuretic, treatments of wounds and Antiinflammatory

Table 1. Species screened for BST.
* Bandoni *et al.* (1972) [16], Jaime *et al.* (1994) [17], Korbes (1995) [18]

Liquid-liquid separation of the crude extract

The crude extracts obtained above were separated by liquid-liquid partitioning using hexane, chloroform, ethyl acetate and water to obtain four fractions for each plant extract with the exception of the *Baccharis* species which were first extracted with $CHCl_3$ and then MeOH to obtain only two fractions for each. All fractions were concentrated to complete dryness.

Brine shrimp lethality test

The extracts, fractions and pure isolated compounds were routinely evaluated in a test for lethality to brine shrimp larvae [3], with minor modifications. Toxicities of compounds were tested at 50, 100, 300, 800 and 1000 ppm in 10 mL sea-water solutions with 1% DMSO (v/v). Ten, one-day nauplii were used in each test and survivors counted after 24 h. Three replications were used for each concentration. A parallel series of tests with the standard potassium dichromate solution (DL_{50} = 20-40 ppm) and the blank control were always conduced. The lethal concentration for 50% mortality after 24 h of exposure, the chronic LC_{50} and 95% confidence intervals were determined using the probit method [10], as the measure of toxicity of the extract or fractions. LC_{50} values greater than 1000 ppm for plant extracts were considered inactive.

RESULTS & DISCUSSION

The extracts studied in this work showed significant lethality against brine shrimp, which has been successfully used as a simple biological test to guide the fractionation process of plant extracts in order to detect antitumour compounds [4]. This bioassay has good correlation with the human solid tumour cell lines [11]. LC_{50} values < 1000 ppm are considered significant for crude extracts [3].

The LC_{50} results of the eleven plant species evaluated in this screening are listed in Table 2. The chloroform extract of the species from genus *Baccharis* were especially active and the chloroform extract from the leaves of *B. pseudotenuifolia* and *B. ligustrina* were the most active among all extracts tested, presenting an LC_{50} of 105 and 115 ppm, respectively. These extracts can be regarded as a promising candidate for a

Species	Part used	Extract	Fraction	LC50 (ppm)	95% CI (ppm)
Baccharis pseudotenuifolia	leaves	$CHCl_3$		105	66 - 186
		MeOH		891	631 - 977
Baccharis ligustrina	leaves	$CHCl_3$		115	105 - 126
		MeOH		912	741 - 1134
Baccharis platypoda	leaves	$CHCl_3$		692	562 - 851
		MeOH		> 1000	
Baccharis coridifolia	leaves	EtOH		832	646 - 891
Polygala paniculata	leaves	EtOH		>1000	
Polygala sabulosa	leaves	EtOH		692	224 - 871
			Hexane	661	467 - 933
			$CHCl_3$	562	398 - 798
			EtOAc	>1000	
			Aqueous	>1000	
Croton celtidifolius	bark	EtOH		>1000	
	leaves	EtOH		676	537 - 692
Cyathea phalerata	wood	EtOH		>1000	
	leaves	EtOH		>1000	
Trichilia catigua	bark	EtOH		>1000	
Eugenia uniflora	leaves	EtOH		>1000	
Schinus molle	leaves	EtOH		>1000	

Table 2. Brine shrimp bioassay results of crude extract and fractions of some Brazilian medicinal plants.

plant-derived antitumour compound. In fact, from these two extracts, oleanolic acid (LC_{50} = 72 ppm), a triterpene, was identified which had its cytotoxic effect confirmed by this bioassay. LC_{50} = 50 - 10 ppm[12] had been reported previously for oleanolic acid. Ethanolic and methanolic extracts from species of the genus Baccharis gave a higher LC_{50} than chloroform extracts, showing that the cytotoxic effect is in the less polar extract.

Solvent partitions of the crude extract of *Polygala sabulosa* were tested for brine shrimp lethality and the results are shown in Table 2. The BST bioassay indicated that the crude extract possessed significant bioactivity, and its LC_{50} was similar to those of the hexane and chloroform fractions. It is possible that a broad range of structurally diverse compounds contribute to the overall pharmacological activity of the crude extract and synergistic effects between active principles may exist. 7-methoxy-6-hydroxy coumarin and styryl-pyrones have been reported as the main components of the chroroform extract of this plant [13]. In previous screening, these constituents and the oil from the hexane fraction showed anti-*Trypanosoma cruzi* activity [14]. These data suggest that the brine shrimp might be used in screening tests suitable for anti-*Trypanosoma cruzi* activity, too. This correlation was observed by Zani *et al.* [15].

The LC_{50} values obtained in the brine shrimp bioassay for *Croton celtidifoilius* shows that the higher effect is presented by leaves extract. These preliminary results indicate that in the leaves there is an active substance that should be isolated by monitoring with BST.

Finally, *Cyathea phalerata, Trichilia catigua, Eugenia uniflora* and *Schinus molle* showed LC_{50} > 1000 ppm, which is considered inactive [3]. These results are of little significance in relation to the cytotoxic activity.

All plant extracts, fractions and pure compounds isolated in phytochemical laboratories should be submitted to as wide a range of bioassays as possible. Numerous plant extracts and isolates stored in phytochemical laboratories have still to be tested and there are certainly many interesting activities yet to be discovered. However, further and more specific bioassays are necessary in order to confirm these conclusions.

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