Revista Brasileira de Ciências Farmacêuticas
Brazilian Journal of Pharmaceutical Sciences
vol. 42, n. 2, abr./jun., 2006

RBCF

Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in Governador Valadares district

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*Ethanol extracts from medicinal plants commonly used by Governador Valadares people were tested for antimicrobial activity and cytotoxicity (BST assay). The field survey was conducted during the years 1997-2000 by means of direct interviews with healing men ("raizeiros") who showed familiarity with local used remedies. A total of 33 crude extracts from 32 plant species was studied. Ten extracts (C*ostus pisonis, Cymbopogon nardus, Eclipta alba, Eleutherine bulbosa, Erigium foetidium, Euphorbia tirucalli, Mikania hirsutissima, Momordica charantia, Solidago microglossa *and* Plectranthus ornatus*) presented brine shrimp toxicity (LD_{50}<1000 ppm). Thirteen extracts presented antimicrobial activity against S*taphylococcus aureus*:* E. alba, Scoparia *sp.*, Arctium lappa, Chammomila tinctoria, E. bulbosa, M. hirsutíssima, S. microglossa, Stachytarpheta dichotoma, Pffafia glomerata, Stenorrhyrchnus lanceolatum, Vernonia condensata *and* Lippia alba. *None extract presented activity against* Escherichia coli.

Uniterms
- Ethnomedicine
- Antimicrobial activity
- *Artemia salina*

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INTRODUCTION

At the Vale do Rio Doce region, where is located Governador Valadares County, Atlantic Forest is almost extinct, and the region is suffering an accelerated desertification process. Besides, in the beginning of Twenty Century, the original indigenous land was occupied by people from several regions of the Country, and the traditional knowledge of that indigenous groups disappeared. Although medicinal plants are becoming extinct by deforestation and interest by the younger generation in learning about the folk medicine is waning, there has been a recent resurgence of the medicinal plants uses in that region. On the other had, few chemical or biological studies about these species have been carried out.

Considering the lack of information and in the aim to rescue some of this folk knowledge, healing men ("raizeiros") from the region were contacted and asked to share with the researchers ethnobotanical information and to guide them collecting botanical material for further studies.

As a result of this study, we present here a list of medicinal plants used in Governador Valadares County, based on healing men information, as well as a

screening about cytotoxicity and antimicrobial activities of these species.

MATERIAL AND METHODS

Plant material

Collection and identification

The field survey was conducted during the years 1997-2000 by means of direct interviews with healing men ("raizeiros") who showed familiarity with local used remedies. Aerial parts of the mentioned plants were collected, under supervision of "raizeiros", from the original vegetation, or from the raizeiro' gardens. Voucher species were deposited at Herbarium of Universidade Vale do Rio Doce, UNIVALE. When possible, plants were growth at Horto Botânico of Universidade Vale do Rio Doce, in the aim to preserve the species.

The plants are listed in Table I in alphabetical order by their scientific name, followed by vernacular (Portuguese) names.

Extract preparation

Aerial parts were oven-dried at temperature under 40 °C, and powdered using knife mill. The powdered material was extracted by maceration using 95% ethanol as solvent at room temperature. The extracts were then filtered and concentrated under vacuum in rotatory evaporator. All the extracts were kept in tightly stoppered bottle in a freezer until used for the biological tests or shared with another research groups.

Antimicrobial activity

The antimicrobial assays were performed by MIC determination and diffusion disc method (Ali-Shtayeh *et al.*, 1998), using *Staphylococcus aureus* (ATCC 25985) and *Escherichia coli* (ATCC 25922). All assays were carried out in triplicate.

Agar diffusion disc method

Briefly, test solution was prepared with known weight of crude extract, dissolved in 200 µL of DMSO. Whatman no. 1 sterile filter paper discs (6 mm) were impregnated with extract (corresponding to 20, 10 and 5 mg/mL of crude plant extract). *In vitro* antibacterial activity was determined by using Mueller Hinton Agar. Petri plates were prepared by pouring 10 mL of Mueller Hinton Agar and allowed to solidify. Plates were dried and 0.1 mL of inoculum suspension was poured and uniformly spread. The discs were then applied and the plates were incubated at 37 °C for 24 h. Each assay in this experiment was repeated thrice. The control assay was performed using ethanol (0.5 mL) and chloramphenicol (20 mg/mL).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was tested by the two-fold serial dilution method. The tested extract was dissolved and was incorporated into 0.5 mL of Mueller Hinton Agar to get a concentration of 20 mg/mL and serially diluted to achieve 10, 5, 2 mg/mL, respectively, and 0.5 mL suspension of the test organism was transferred on to each tube. The negative control tube contained only organisms and not the plant extract. The positive control tube contained only chloramphenicol (2 mg/mL). The culture tubes were incubated at 37 °C for 24 h. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation was determined as MIC.

Minimum bactericidal concentration (MBC)

To confirm the activity, all the tubes used in the MIC study which did not show any growth of the bacteria after the incubation period were first diluted (1:4) in fresh Mueller Hinton Broth and then subcultured on to the surface of the freshly prepared Mueller Hinton Agar plates and incubated at 37 °C for 24 h. The MBC were recorded as the lowest concentration of the extract that did not permit any visible bacteria colony growth on the appropriate agar plate after the period of incubation.

Brine shrimp lethality test (BST)

The assay was performed basically according to simplified Meyer's method (Meyer *et al.,* 1982). Briefly, brine shrimp *Artemia salina* L. encysted eggs (Maramar) were incubated in artificial seawater at 28 °C. Samples were solved in 20 mL of artificial seawater. Serial dilutions (triplicate) were prepared in the same solution. Metanauplii (10 units) were added to each set of tubes containing samples and the cultures further incubated for 24 h. Controls containing only artificial seawater were included on set of experiment. Lapachol was used as reference standard. LC_{50} (after 24 h) was calculated by Probit analysis.

RESULTS AND DISCUSSION

The results of the screening of crude plant extracts about antimicrobial and brine shrimp toxicity (BST) activities are presented in Tables II and III, respectively.

Meyer *et al*. (1982) classified crude extracts into toxic

TABLE I - Ethnobotanical data on medicinal species used in Governador Valadares District

Voucher number	Species	Common names	Used parts	Ethnomedicinal uses
		Amaranthaceae		
726	*Alternanthera dentata* (Moench) Stuchlik	Doril	Aerial parts	Anti-inflammatory, analgesic
727	*Alternanthera dentata* (Moench) Stuchlik – var. *alba*	Terramicina	Aerial parts	Antibiotic
315	*Alternanthera tenela* Colla	Apaga-fogo	Aerial parts	Diuretic
037	*Pfaffia glomerata* (Spreng) Pedersen	Ginseng-brasileiro, acônito-do-mato, para-tudo, anador,	roots	Anti-flogistic, analgesic, anti-cold, antibiotic
		Apiaceae		
756	*Erygium foetidum* L.	Coentro-bravo	Aerial parts	Spice; abortion induction and in sexual dysfunctions, diarrhoea, fever, headaches
		Asteraceae		
379	*Acanthospermum hispidum* DC	Benzinho	Aerial parts	Anti-tossing; pneumonia; venereal diseases,
		Asteraceae		
012	*Artemisia absinthium* L.	Losna	Aerial parts	Diuretic; vermifugue
053	*Chamomilla tinctoria* L.	Camomila		Colic; flatulence
625	*Eclipta alba* (L.) Hansk	Lanceta, erva-botao, agriao-do-brejo, sucuruina, erva-lanceta	Aerial parts	Anti-hepatotoxic, anti-asthma, skin diseases, against snake bites, depurative
304	*Arctium lappa* L.	Bardana	Aerial parts	Depurative; diuretic
647	*Mikania hisutissima* DC.	Cipo-cabeludo	inflorescence	Lumbar pain; renal diseases
763	*Solidago microglossa* DC. Sin: *S. vulneraria, S. odora S. marginella, S. nitidula, S. polyglossa,*	Arnica do Brasil, erva-lanceta, sape-macho, lanceta, arnica-silvestre, pronto-alívio	Aerial parts	Anti-rheumatism, analgesic, anti-inflammatory, ear pain, vulnerary
		Borraginaceae		
700	*Heliotropium indicum* L.	Morrainha	Aerial parts	Anti-hemorroidal

TABLE I - (cont.) Ethnobotanical data on medicinal species used in Governador Valadares District

Voucher number	Plant name	Vernacular names	Used parts	Ethnomedicinal uses
		Brassicaceae		
644	*Nasturtium* sp	Agrião	Aerial parts	Carminative
		Convolvulaceae		
343	*Ipomoea cairica* (L.) Sweet.	Corda-de-viola	Aerial parts	Purgative, analgesic, anti-inflammatory, anti-rheumatism
333	*Ipomoea quamoclit* L.	Cipó-esqueleto	Aerial parts	Antibiotic
744	*Ipomoea alba* L.		Aerial parts	Anti-pyretic, hipotensive, emollient, to treat headache
		Costaceae		
109	*Costus pisonis* L.	Cana-de-macaco	Aerial parts	Anti-inflammatory
		Curcubitaceae		
061	*Momordica charantia* L.	Melão-de-são-caetano	Aerial parts	Anti-flogistic; colic; diabetes
		Euphorbiaceae		
245	*Euphorbia tirucalli* L.	Aveloz,	Aerial parts	To cure warts; anti-rheumatism, anti-cancer
1503	*Atropa multifera* L.	Mertiolate	Leaves	To heal wounds
		Iridaceae		
198	*Eleutherine bulbosa* (Mill.) Urb.	Ruibarbo-do-campo	Leaves Bulb	Purgative, anti-cancer
		Lamiaceae		
092	*Ocimum americanum* L.	Manjericão-branco, manjericão, alfavaca-de-vaqueiro, basilicão	Aerial parts	Stimulant, carminative, bile secretion
087	*Plectranthus barbatus* Andrews Sin: *Coleus barbatus*	Boldo	Leaves	Stomach diseases
100	*Plectranthus ornatus* Codd. (*P. neochilus* Schlechter)	Boldinho	Leaves	Anti-pyretic, diuretic, bile secretion, astringent, analgesic, depurative, anti-inflammatory, pectoral, anti-oedema, anti-ulcer, antibiotic

TABLE I - (cont.) Ethnobotanical data on medicinal species used in Governador Valadares District

Voucher number	Plant name	Vernacular names	Used parts	Ethnomedicinal uses
		Orchidaceae		
937	*Cyrtopodium* sp	Sumare	Pseudo-bulb	Topic antibiotic
978	*Stenorrhychnus lanceolatum* (Aubl.)	Salsa largata	Root	Aphrodisiac
		Poaceae		
832	*Cymbopogon nardus* (L.)Rendle	Citronela	Leaves	Insecticide
		Plantaginaceae		
143	*Plantago major* L.	Tanchagem, tancha, trancagem, tanchagem maior	Leaves	Anti-pyretic, anti-pectoral, anti-inflammatory, anti-oedema, antibiotic analgesic, diuretic, depurative, bile secretion, astringent, anti-ulcer
		Portulacaceae		
671	*Talinum pattens* (Jacq.) Willd.	Lingua-de-vaca, piolho	Aerial parts	Anti-pyretic, hypotensive, emollient, to treat headache
		Verbenaceae		
605	*Stachytarpheta dichotoma* (Ruiz & Pav.) Vahl.**Sin:** *S. gibberosa, S. jamaicensis, S. umbrosa, S. cayamnensis, Verbena jamaicensis, V. dichotoma, Cymburus urticifolia polyglossa, S. vulneraria, S. odora*	Mocotó, gervão-roxo, erva-gervão, verbena-falsa, gerbão, gervão-de-folha-de-verônica	Aerial parts	Tonic, anti-stomach aches, renal diseases, antiseptic, cholagogic, anti-spasmodic, anti-pyretic, anti-ulcerative, gastric protector, anti-cold
		Violaceae		
912	*Anchieta salutaris* L.	Cipó-suma; suma-roxa, piragaia	Roots	Purgative, anti-rheumatism, antibiotic, depurative
		Vitaceae		
1340	*Cissus sicyoides* L.	Insulina vegetal	Fruits; seeds	Anti-rheumatism, convulsive; anti-inflammatory; antibiotic; hypoglicemic, anti-epilepsy

TABLE II - Antimicrobial activity of ethanol extracts medicinal plants from Governador Valadares, Brazil

Plants	Ethanol extract yield (%)	Disk (mg.mL^{-1})*		MIC (mg mL^{-1}) *	
		S.a	E.c.	S.a	E.c.
Arctium lappa	11.7	R	R	5	R
Chammomila tinctoria	4.3	*20*	*R*	*5*	*R*
Eclipta alba	6.35	R	R	1	R
Eleutherine bulbosa (bulb)	23.0	*5*	*R*	*5*	*R*
Lippia alba	8.1	R	R	20	R
Mikania hirsutíssima	14.9	*20*	*R*	*5*	*R*
Pffafia glomerata	7.91	10	R	10	R
Plectranthus ornatus	6.1	5	*R*	*R*	*R*
Scoparia sp.	28.6	10	R	2	R
Solidago microglossa	21.4	R	R	5	R
Stachytarpheta dichotoma	13.7	R	R	5	R
Stenorrhyrchnus lanceolatum	29.3	*R*	*R*	*10*	*R*
Vernonia condensata	10.7	R	R	10	R
Chloramphenicol		20	20	2	2

* = concentration that promote inhibition zone; R= inactive; S.a.=*Staphylococcus aureus; E.c.=Escherichia coli*

(LC_{50} < 1000 ppm) and non-toxic (LC_{50} >1000 ppm), according to the levels required to attain the LC_{50} in the BST assay. Based on this classification, ten extracts (*C. pisonis*, *C. nardus*, *E. alba, E. bulbosa, E. foetidium*, *E. tirucalli, M. hirsutíssima*, *M. charantia, S. microglossa* and *P. ornatus*) presented BST (LD_{50}< 1000 ppm). In addition, ethanol extract of *E. tirucalli, M. charantia* and *P. ornatus* presented LD_{50} values lower than lapachol (positive control).

The extracts were considered to have antimicrobial activity when presented inhibition halo bigger or equal that promoted by chloramphenicol (20 mg/mL chloramphenicol promoted 25 mm inhibition halo, measured by the center of the disc). Then, thirteen extracts presented antimicrobial activity against *S. aureus*: *E. alba (S*>1 mg/mL); *Scoparia* sp. *(S*>2 mg/mL); *A. lappa, C. tinctoria, E. bulbosa*, *M. hirsutíssima, S. microglossa e S. dichotoma (S*>5 mg/mL); *P. glomerata*, *S. lanceolatum, V. condensata (S*>10 mg/mL); *L. alba (S*>20 mg/mL). None presented activity against *E. coli*.

Although some species are used in folk medicine as antibiotic or depurative, at the assay conditions, no antimicrobial activity was detected: *Alternanthera dentata* var. alba (terramicina), *Acanthospermum hispidum* (benzinho), *Ipomoea quamoclit* (cipó-esqueleto), *Cyrtopodium* sp (sumaré), *Plantago major* (tanchagem), *Stachytarpheta dichotoma* (gervão-roxo), *Anchieta salutaris* (suma-roxa) and *Cissus sicyoides* (insulina vegetal), all of them nominated by "raizeiros" as antibiotic or depurative remedies, did not present such activity. On the other hand, species without ethnomedicinal report about antibiotic activity inhibited bacteria growth. Curiously, *Chamomilla tinctoria*, which inhibit *S. aureus* growth, was not nominated as an antibiotic remedy by "raizeiros", despite several reports at literature present extracts from *Chamomilla* species as an antimicrobial agent (Benetti, Manganelli, 1985; Lu *et al.,* 1998; Stamatis *et al*., 2003; Mazokopakis *et al*., 2005).

Bara and Vanetti (1998) tested extracts from *A. lappa* and *P. major* against *S. aureus* and those extracts did not present activity. In another work, Freitas *et al*. (2002) showed that the hydro-ethanol extract obtained from leaves of *P. major* was effective against *S. aureus*. Despite Pessini *et al*. (2003) had been tested *Lippia alba* ethanol extract and

TABLE III - Toxicity of medicinal plants from Governador Valadares, Brazil, against *Artemia salina* brine shrimp

plant	LD_{50} [a] (ppm)	RT [b] (%)
lapachol (control)	59.17	100.0
Costus pisonis	389.26	15.2
Cymbopogon nardus	118.92	50.0
Eclipta alba	352.92	16.8
Eleutherine bulbosa (bulb)	269.59	21.9
Erygium foetidium	885.37	6.7
Euphorbia tirucalli	23.10	256.1
Mikania hirsutíssima	634.84	9.3
Mormordica charantia	41.33	143.2
Plectranthus ornatus	31.93	185.3
Solidago microglossa	72.42	81.7

[a] LD_{50} : Lethal doses at 50% population; [b] RT: relative toxicity in comparison with lapachol.

without finding activity, the sample tested by our group presented *S. aureus* inhibition at concentration of 5 mg/mL.

A total of 33 plant extracts from 32 different plant species (one with two varieties) belonging to 9 families were studied. Table I presents the botanical names and voucher specimens numbers, the vernacular names and their uses in traditional medicine, as known according to information mainly collected by MSc Beatriz Gonçalves Brasileiro through interviews with local traditional healers.

The fairly good degree of correlation of traditional medicine claims with the biological activities as observed in the present preliminary study results, warrant further investigation. Activity guided fractionation and phytochemical investigation of some species are already underway by our group.

From *Plectranthus ornatus* have been isolated several diterpenes and triterpenes (Oliveira *et al*., 2005; Oliveira, 2002). *Ipomoea cairica* presented antinociceptive activity *in vivo* (Ferreira *et al.,* 2005). From ethanol and hydro-ethanol extracts of *I. cairica* were isolated lignans, triterpenes, and caffeic acid derivatives (Ferreira *et al*., 2005). Preliminary studies showed *Euphorbia tirucalli* ethanol extract induces an increase on human peripheral blood mononuclear cells (PBMC) proliferative response (Malaquias *et al*., 2000).

RESUMO

Triagem das atividades antimicrobiana e citotóxica de algumas plantas medicinais brasileiras usadas na cidade de Governador Valadares

Os extratos etanólicos de plantas medicinais utilizadas por moradores da cidade de Governador Valadares foram avaliados quanto às atividades antimicrobiana e citotóxica. A pesquisa de campo foi realizada durante o período de 1997-2000, por meio de entrevistas com os raizeiros locais. Foram avaliados 33 extratos brutos de um total de 32 espécies. Desses extratos, dez apresentaram toxicidade às larvas de Artemia salina *(DL_{50}<1000 ppm):* Costus pisonis, Cymbopogon nardus, Eclipta alba, Eleutherine bulbosa, Erigium foetidium, Euphorbia tirucalli, Mikania hirsutissima, Momordica charantia, Solidago microglossa e Plectranthus ornatus. *Quanto à atividade antimicrobiana, nenhum dos extratos apresentou atividade contra* Escherichia coli. *Entretanto, treze extratos mostraram-se ativos contra* Staphylococcus aureus*:* E. alba, Scoparia sp., Arctium lappa, Chammomila tinctoria, E. bulbosa, M. hirsutíssima, S. microglossa, Stachytarpheta dichotoma, Pffafia glomerata, Stenorrhyrchnus lanceolatum, Vernonia condensata *e* Lippia alba.

UNITERMOS: Etnomedicina. Artemia salina. *Atividade antimicrobiana.*

ACKNOWLEDGEMENTS

The authors are indebted with raizeiros for informations and Fundação Percival Farqhuar, Governador Valadares, Brazil for financial support.

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Recebido para publicação em 12 de maio de 2005.
Aceito para publicação em 16 de março de 2006.