Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Hernando Herrera-Mata, Alfredo Rosas-Romero & Oscar Crescente V (2002) Biological Activity of "Sanguinaria" (Justicia secunda) Extracts, Pharmaceutical Biology, 40:3, 206-212, DOI: 10.1076/phbi.40.3.206.5826

To link to this article: https://doi.org/10.1076/phbi.40.3.206.5826

Published online: 29 Sep 2008.

Pharmaceutical Biology
2002, Vol. 40, No. 03, pp. 206–212

1388-0209/02/4003-206$16.00

Biological Activity of "Sanguinaria" (*Justicia secunda*) Extracts

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Abstract

A biological screening of the activity of *Justicia secunda* Vahl. extracts and fractions against several Gram-positive and Gram-negative bacterial strains of Enterobacteriaceae and in the brine shrimp *Artemia salina* Linn. assay, is reported. The ethanol extract of the aerial parts of the plant was sequentially partitioned with *n*-hexane (FHX) and chloroform (FCH), remaining a water solution (FOH). All fractions showed bactericidal activity against Gram-positive bacteria *Bacillus cereus* (ATCC 9634) and *Staphylococcus aureus* (ATCC 6538P), while they were inactive against Gram-negative strains *Escherichia coli* (ATCC 0389), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 9920) and *Salmonella typhimurium* (ATCC 14028). The water-soluble fraction (FOH) showed activity in the brine shrimp bioassay, yielding a LC_{50} value of 37.93 µg/ml. The original FHX and FCH fractions were subjected to short column chromatography. Three fractions from FHX and four fractions from FCH were active against Gram-positive bacterial strains, and they were inactive against the Gram-negative ones.

Keywords: Antibacterial activity, *Artemia salina* Linn., ethnobotanic use, *Justicia secunda* Vahl., LC_{50} value, toxicity test.

List of the Latin binomial of all organisms noted in the body of the manuscript (in order of citation):

Justicia secunda Vahl. (Acanthaceae)
Justicia procumbens Linn. (Acanthaceae)
Justicia pectoralis Jacq. (Acanthaceae)
Aedes aegypti Linn. (Culicidae)
Justicia simplex D. Don (Acanthaceae)
Justicia prostrata Schlecht. ex Nees (Acanthaceae)
Justicia flava Vahl. (Acanthaceae)
Justicia gendarussa Burm. (Acanthaceae)
Justicia glauca Rottl. (Acanthaceae)
Justicia ghiesbreghtiana Nees (Acanthaceae)
Justicia tranquebariensis Linn. (Acanthaceae)
Justicia hyssopifolia Kunth. (Acanthaceae)
Justicia spicigera Schlecht. (Acanthaceae)
Giardia duodenalis Davis (Polymastigida)
Justicia insularis T. Anderson (Acanthaceae)
Justicia ciliata V.A.W. Graham (Acanthaceae)
Justicia extensa T. Anderson (Acanthaceae)
Tilapia nilotica Linn. (Cichlidae)
Artemia salina Linn. (Anostraca)
Bacillus cereus Frank. & Frank. (Enterobacteriaceae)
Staphylococcus aureus Rosenbach (Enterobacteriaceae)
Escherichia coli T. Esch. (Enterobacteriaceae)
Klebsiella pneumoniae E. Klebs. (Enterobacteriaceae)
Proteus vulgaris Hauser (Enterobacteriaceae)
Salmonella tiphymurium D.E. Salmon (Enterobacteriaceae)
Pseudomonas aeruginosa Schroeter (Pseudomonaceae)
Candida albicans Langenbeck (Cryptococcaceae)

Introduction

Several species of the genus *Justicia* (Acanthaceae) are reported in the traditional medicine of some countries. *Justicia secunda* Vahl., well known in the Venezuelan folk medicine as "Sanguinaria", grows in humid soils forming colonies beside rivers and creeks. It is an erect grass of simple leaves, whose flowers are contained in terminal panicles (Heywood & Moore, 1978). It is distributed in the north of South America; in Venezuela it is found in the north-eastern part of the country, where the boiled water extract of the aerial parts is used as antipyretic, sanguineous depurative and in the treatment of the chicken pox (Schnee, 1984); while

Accepted: December 13, 2001

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in other countries infusions of aerial and entire plant are used in treatment of diabetes (Mahabir & Gulliford, 1997) and amenorrhea (Wong, 1976).

Some work has been done on the secondary metabolites of several species of the genus *Justicia*. One of the most studied, *J. procumbens* Linn., is used in the traditional medicine as antipyretic and against laryngitis; from this plant for the first time neojusticin-A was isolated (Okigawa et al., 1970a), a compound with significant inhibitory activity of the platelet aggregation (Chen et al., 1996). Neojusticin B and taiwanin E (Okigawa et al., 1970b), as well as the justicidins C and D (Ohta & Munakata, 1970) were also isolated. The lignan neojusticin B induced antiarrhytmic activity in rats (Lin et al., 1982). Also, in India, the anthocyanin peonidin 3-glucoside was isolated from this species (Tiwari et al., 1978), plus two cytotoxic compounds, justicidin-A and diphyllin (Fukamiya & Lee, 1986), that, in a later study, were demonstrated to be potent antivirals (Asano et al., 1996). In recent years, from this same species, six new diarylbutane lignans have been isolated, although no biological activity was reported (Chen et al., 1998).

Another species with several reports in the literature is *J. pectoralis* Jacq., which is broadly used to alleviate coughing and as a cure for lung infections (Mills et al., 1986); from this plant justicidin-B has been isolated, a cytotoxic principle with proven *in vivo* piscicidal activity (Okigawa et al., 1970b) and *in vitro* antileukemic activity (Joseph et al., 1988). This plant is also the main ingredient in the hallucinogenic snuff of some South American indigenous tribes (MacRae & Towers, 1984; de Smet, 1985). Of the extracts of this plant, betaine, coumarin and umbelliferone have been isolated, all them with varied biological activity (MacRae & Towers, 1984). *O*-Methoxylated-C-glycosylflavones (Joseph et al., 1988) and several coumarins and derivatives of 3-phenylpropionic acid (de Vries et al., 1988) have been isolated and reported. On the other hand, the alcoholic extract of *J. pectoralis* Jacq. exhibited *in vivo* insecticide properties against the larvae of the dengue transmitting mosquito *Aedes aegypti* Linn. (Chariandy et al., 1999). Recently, the bronchodilator activity of extracts of this specie on guinea pig has been proved (Leal et al., 2000).

From *J. simplex* D. Don there has been isolated: simplexolin, sesamin, asarinin, sesamolin and sitosterol (Ghosal et al., 1979a); 3-arylnaphthalide lignans helioxanthin, justicin C and justicidin E (Sastry et al., 1979); the furofuran lignans, justisolin and simplexoside, both with biological properties (Ghosal et al., 1980); and additionally a triterpenoidal saponin, justicisaponin-I, a potent antifertility agent in female rats (Ghosal et al., 1981). Likewise, *J. prostrata* Schlecht. ex Nees yielded retrochinensin lignan (Ghosal & Banerjee, 1979), and the prostalidins A, B and C, the first of which showed antidepressive activity in rats and albino mice (Ghosal et al., 1979b).

Another species, *J. flava* Vahl., has been the object of phytochemical studies, yielding the lignans helioxanthin, (+)-isolariciresinol and justicinol (Olaniyi & Powell, 1980), and β-sitosterol, stigmasterol and campesterol (Olaniyi, 1980).

From a Hindu species, *J. gendarussa* Burm., β-sitosterol has been isolated (Wahi et al., 1974), and aromatic amines (Chakravarty et al., 1982).

The furanoid lignan justiciresinol (Subbaraju et al., 1991) and several other lignans, among them jusglaucinol (Rajendiran et al., 1991), have been isolated from *J. glauca* Rottl.

In *J. ghiesbreghtiana* Nees there have been identified derivatives of α-malamic acid (Ismail et al., 1998), and an amide of L-threo-γ-hydroxyglutamic acid (Lorenz et al., 1999).

Starting from the aerial parts of *J. tranquebariensis* Linn. the lignans aryltetralin, (+)-lariciresinol and (+)-medioresinol were isolated (Raju & Pillai, 1989). Another lignans, denominated J1 and J2, this last one a β-D glucoside, were found in *J. hyssopifolia* Kunth. (Trujillo et al., 1990).

The acidified alcoholic extract of *J. spicigera* Schlecht. showed activity against the intestinal parasite *Giardia duodenalis* Davis, Polymastigida (Ponce-Macotela et al., 1994). Kaempferitrin and *bis*-rhamnoside (Euler & Alam, 1982), along with *O*-sitosterol-3β-glycoside, allantoin and cryptoxantin (Domínguez et al., 1990) were reported in this species.

The aqueous extract of *J. insularis* T. Anderson, which is used to regulate the menstrual cycle and to treat the dysmenorrhoea and infertility, has proven to be effective in the regulation of the reproductive parameters in immature female rats (Telefo et al., 1998). Several lignans from *J. ciliata* V.A.W. Graham with cytotoxic activity against tumor cells *in vitro*, were isolated (Day et al., 1999); while the boiled aqueous extract of *J. extensa* T. Anderson has proven to be highly toxic in the bioassay against the fish *Tilapia nilotica* Linn., Cichlidae (Ibrahim et al., 2000).

In spite of the importance of the genus and widely use of the species *J. secunda* Vahl. in the Venezuelan traditional medicine, there are no reports of ethnobotanical and phytochemical studies on that plant. In this paper we present the results of our study of the antimicrobial and cytotoxic properties of fractions, obtained by liquid/liquid partition (with hexane and chloroform) of an ethanol extract of the aerial parts of the plant; and of the subfractions resulting from short column chromatography of those fractions. We also present the identification, by GC/MS, of the most abundant compounds in the fractions or subfractions with relevant activity.

Materials and methods

Plant material

Justicia secunda Vahl. was collected in the town San Juan of Macarapana, near Cumaná (estado Sucre, Venezuela). The confirmation of the species was carried out by taxonomic studies and by comparison with a sample deposited at the Isidro Bermúdez Herbarium of the Universidad de Oriente (UDO, Cumaná), registered with the code LC-5202/91.

Preparation of plant extracts

The collected vegetable material, consisting of aerial parts of the plant (stems and leaves) was dried, for one week, at ambient temperature in a dry and fresh place. A total of 653.6 g of dry vegetable material was finely milled and extracted with absolute ethanol (3 l), for 5 days, at room temperature. The solvent was removed *in vacuo* on a Büchi 121 rotavapor, until about 150 ml. This ethanol extract was diluted with distilled water (500 ml) and allowed to rest, at 4 °C, for 5 days. After removing most of the chlorophyll by decantation, the aqueous phase was successively extracted with *n*-hexane (4 × 250 ml), and then with chloroform (4 × 250 ml). The organic fractions were concentrated *in vacuo*, while the remaining aqueous solution fraction was lyophilized (Freeze Dryer 18, Labconco). At the end of the partition procedure we obtained 1.31 g of the *n*-hexane fraction (FHX), 2.64 g of the chloroform fraction (FCH) and 13.27 g of the remaining lyophilized aqueous solution (FOH).

Chromatographic procedure

Original fractions FHX and FCH were subjected to short column chromatography (Rigby & Hunt, 1967) using silica gel 60 (Merck, 35–70 mesh) and eluted with a gradient of solvents, *n*-hexane, chloroform, ethyl acetate and methanol in several proportions. Starting from FHX, 16 eluates of approximately 5 ml each were obtained, which were pooled into 6 fractions (SFHXi) after Rf's comparison by thin layer chromatography (TLC), using silica gel supported on aluminum sheets (Merck 60 F_{254}). Solvents were removed from the fractions and were ready for the antibacterial bioassays. The same chromatographic procedure was applied to the chloroform fraction (FCH), obtaining in this case 11 fractions for antibacterial bioassays (SFCHi), from 25 eluates obtained. FOH was bioassayed without further fractionating.

Bioassays

Two types of biological tests were selected: Antibacterial tests in Petri dishes (Bauer et al., 1966) and toxicity assays against brine shrimp *Artemia salina* Linn., Crustacea, Anostraca (Meyer et al., 1982). For the antibacterial test, we prepared solutions (15 mg/ml) of FHX and FCH in chloroform and of FOH in distilled water at the same concentration. Then, Whatman No. 3 filter paper disks (Ø = 7 mm) were impregnated with 12 µl of the each above prepared solutions. The same procedure was employed for the fractions obtained from FHX and FCH, only in this case the concentration was 10 mg/ml. All assays were run in triplicate and the inhibition halos were measured (in mm) after incubation by 24 h at 37 °C. The chromatographic conditions of the fractions that were bioactives are represented in Table 3.

All the bacterial strains used in the antibacterial bioassays belong to the family Enterobacteriaceae. The Gram-positive bacterial strains used were: *Bacillus cereus* Frank. & Frank. (ATCC 9634) and *Staphylococcus aureus* Rosenbach (ATCC 6538P); while the Gram-negative ones were: *Escherichia coli* T. Esch. (ATCC 0389), *Klebsiella pneumoniae* E. Klebs. (ATCC 10031), *Proteus vulgaris* Hauser (ATCC 9920) and *Salmonella typhimurium* D.E. Salmon (ATCC 14028). The microorganisms were cultured in Müeller-Hinton culture media (Difco DF0252-17-6). As a positive control, commercial antibiotics were used (BBL-sensi-disc), and as a negative control, paper disks (Whatman No. 3) were impregnated with 12 µl of *n*-hexane or chloroform.

For the toxicity test on FOH, serial aqueous dilutions of this extract were used (1000 to 0.1 ppm), in triplicate. The naupliis of brine shrimp *A. salina* were obtained by eclosion of their eggs in a prepared saline solution with synthetic marine salt (Instant Ocean of Aquarium Systems, Mentor, Ohio) in distilled water, with a final concentration of 38 ppm. For the toxicity test in *A. salina* we used the fraction FOH for its solubility in the saline solution where the naupliis of this brine shrimp eclosionated, while the fractions FHX and FCH were not used because they were not soluble in the mentioned solution.

GC/MS analysis

The fractions coming from FHX and FCH original fractions, and those that were active in the antibiogram test, were analyzed by gas chromatography/mass spectroscopy (GC/MS), using a GC HP 5890 series II, coupled to a quadrupole mass selective detector HP 5971A. The resulting mass spectra were compared with those in a commercial database Wiley 138L.

Results and discussion

As expected, the diameters of inhibition halos exhibited by the well-known commercial antibiotics (Table 1) were bigger than those shown by the crude extract (FOH), original fractions (FHX and FCH) and fractions that were obtained of them (Tables 2, 4 and 5), because the used drugs are pure and they have well-known antibacterial activities. For example, chloramphenicol, erythromycin and tetracycline inhibit the cytoplasmic proteins synthesis, while ampicilin inhibits cellular wall formation (Avendaño, 1993).

Table 2 presents the results of the antibacterial activity of FHX, FCH and FOH. As we can observe, all the fractions and subfractions produced inhibition of the bacterial growth in the Gram-positive strains but not of the Gram-negative ones. The bactericidal activity exhibited by all the samples on *B. cereus* and *S. aureus* is important, because these microorganisms are responsible for gastrointestinal affections and, particularly in the case of *S. aureus*, which it produces an enterotoxin (toxin of the syndrome of shock toxic, TSST-1), responsible for fever, shock states and desquamative cutaneous eruption in human skin (Jawets et al., 1976). It is important to highlight that in a previous study with several species of the families Scrophulariaceae and

Table 1. Sensitivity of tested bacteria against commercial antibiotics.

Bacterial strains	Antibiotics			
	Ampicilin (10 µg)	Chloramphenicol (30 µg)	Erythromycin (15 µg)	Tetracycline (30 µg)
Gram-positives				
Bacillus cereus ATCC 9634	–	25	29	31
Staphylococcus aureus ATCC 6538P	–	–	26	–
Gram-negatives				
Escherichia coli ATCC 0389	19	–	–	–
Klebsiella pneumoniae ATCC 10031	24	–	–	–
Proteus vulgaris ATCC 9920	–	15	14	–
Salmonella typhimurium ATCC 14028	–	23	24	26

Diameters of inhibition halos in mm, at given concentrations of commercial antibiotics. – = No activity.

Table 2. Antibacterial activity of original fractions obtained from the aerial parts of *Justicia secunda*.

Bacterial strains	*J. secunda* fractions		
	n-Hexane (FHX)	Chloroform (FCH)	Aqueous (FOH)
Gram-positives			
Bacillus cereus ATCC 9634	16	21	14
Staphylococcus aureus ATCC 6538P	14	24	29
Gramnegatives			
Escherichia coli ATCC 0389	–	–	–
Klebsiella pneumoniae ATCC 10031	–	–	–
Proteus vulgaris ATCC 9920	–	–	–
Salmonella typhimurium ATCC 14028	–	–	–

Diameters of inhibition halos in mm, concentration = 15 mg/ml. – = No activity.

Acanthaceae (Meurer-Grimes et al., 1996), in which *J. secunda* was selected, methanol crude extracts of aerial parts of this plant showed no bioactivity against the bacteria *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* Schroeter, Pseudomonaceae and the yeast *Candida albicans* Langenbeck, Cryptococcaceae, that which contrasts with some results obtained by us, since all our extracts and fractions obtained from *J. secunda* were bioactives against *S. aureus*.

Table 3. Fractions obtained by short column chromatography of FHX and FCH with activity in the antibacterial tests.

Fractions	Origin of fractions	Solvent system
Codes:	**From *n*-hexane fraction (FHX)**	
SFHX4		*n*-Hexane : Chloroform (4 : 1)
SFHX6		*n*-Hexane : Chloroform (2 : 1)
SFHX10		Chloroform : Ethyl Acetate (4 : 1)
Codes:	**From chloroform fraction (FCH)**	
SFCH1		*n*-Hexane : Chloroform (9 : 1)
SFCH6		Chloroform : Ethyl Acetate (4 : 1)
SFCH7		Chloroform : Ethyl Acetate (1 : 3)
SFCH8		Ethyl Acetate : Methanol (5 : 1)

Table 4. Antibacterial activity of three fractions obtained from original *n*-hexane fraction (FHX) of *J. secunda*.

	Fractions		
Bacterial strains	SFHX4	SFHX6	SFHX10
Gram-positives			
Bacillus cereus ATCC 9634	11	13	15
Staphylococcus aureus ATCC 6538P	13	15	14
Gram-negatives			
Escherichia coli ATCC 0389	–	–	–
Klebsiella pneumoniae ATCC 10031	–	–	–
Proteus vulgaris ATCC 9920	–	–	–
Salmonella typhimurium ATCC 14028	–	–	–

Diameters of inhibition halos in mm, concentration = 10 mg/ml. – = No activity.

Table 5. Antibacterial activity of four fractions obtained from original chloroform fraction (FCH) of *J. secunda*.

	Fractions from FCH			
Bacterial strains	SFCH1	SFCH6	SFCH7	SFCH8
Gram-positives				
Bacillus cereus ATCC 9634	13	16	17	18
Staphylococcus aureus ATCC 6538P	16	22	21	17
Gram-negatives				
Escherichia coli ATCC 0389	–	–	–	–
Klebsiella pneumoniae ATCC 10031	–	–	–	–
Proteus vulgaris ATCC 9920	–	–	–	–
Salmonella typhimurium ATCC 14028	–	–	–	–

Diameters of inhibition halos in mm, concentration = 10 mg/ml. – = No activity.

In Table 4, the antibacterial activities of three bioactive fractions (SFHX4, SFHX6 and SFHX10), obtained by column chromatography of the *n*-hexane fraction are shown, while the equivalent data of four chloroform ones (SFCH1, SFCH6, SFCH7 and SFCH8) are shown in Table 5. As in Table 2, all fractions were bactericidal against the Gram-positive strains only, which is obvious because these subfractions came from samples that did not exhibit activity against Gram-negatives ones. As it is known, the structure of the cellular wall of the Gram-negative bacteria is more complex than of the Gram-positives ones because, besides presenting the rigid structure of peptidoglycan common to both groups of microorganisms, the Gram-negatives microorganisms present a double cellular membrane, external and internal membrane, with a space among them in which numerous enzymes exist (β-lactamase, for example) and their activity confer these bacteria resistance to certain antibiotics (Jawets et al., 1976; Avendaño, 1993).

The analysis of the seven bioactive fractions, by GC/MS, and the subsequent comparison of the obtained spectra with those of the database Wiley 138.L are detailed next. The three bioactive fractions from the *n*-hexane fraction (FHX), showed a mixture of hydrocarbons and fatty acids of long chain. Subfractions SFHX4 and SFHX6 were constituted

Table 6. Toxicity of extract FOH of *Justicia secunda* against *Artemia salina.*

Vegetable material	Crude extract	LC_{50} value	95% Confidence limits
Aerial parts	Aqueous	37.93	12.62–126.01

LC_{50} value in µg/ml.

mainly by neophytadiene, hentriacontane and decanoic acid, while SFHX10 was formed for the most part by hexadecanoic and octadecanoic acids.

On the other hand, the four bioactive fractions from the chloroform fraction (FCH) were constituted for the most part by fatty acids, terpenoids and carboxylic acids. Fractions SFCH1 was constituted mainly by hexadecanoic and octadecanoic acids; SFCH6 and SFCH7 were formed more than of the total by a mixture of octadecanoic, 9-octadecenoic and eicosanoic acids methyl esters, while fraction SFHX8 was composed by 11-hexadecenoic acid methyl ester, taraxasterol and squalene as majority compounds.

No antibacterial activity was observed on Petri dishes with both the negative controls (*n*-hexane and chloroform).

With regard to the toxicity test in *A. salina* on FOH (Table 6), we obtained a value for LC_{50} equal to 37.93 µg/ml, calculated by the Probit method (Finney, 1971). This small crustacean has been used extensively in toxicity bioassays for monitoring the presence of toxic and potentially useful antitumoral compounds. According to the literature (Meyer et al., 1982; Ojala et al., 1999), an extract is considered potentially useful as cytotoxic, when its LC_{50} is around 30 µg/ml, which is our case.

According to our results, there is a justification for the traditional use of *J. secunda* in the treatment of gastrointestinal disorders, due to their inhibitory activity on the enterobacteriaceae *B. cereus* and *S. aureus*. Moreover, the result obtained in the cytotoxicity test is very interesting, since it would be another ethnobotanical property that could be exploited in this plant. It will be necessary to carry out other antiviral tests to check the claimed activity of extracts of this plant in the treatment of chicken pox (Schnee, 1984).

Acknowledgements

The authors wish to thank Professor Luis José Cumana, taxonomist of the Herbarium of Universidad de Oriente, for his collaboration in the botanical identification of the plant used in this work.

References

Asano J, Chiba K, Tada M, Yoshii T (1996): Antiviral activity of lignans and their glycosides from *Justicia procumbens* L. *Phytochemistry 42*: 713–717.

Avendaño LM (1993): *Introducción a la química farmacéutica*. Madrid, España. McGraw-Hill Interamericana, pp. 313–318.

Bauer A, Kirby W, Sheris I, Turk M (1996): Antibiotic susceptibility testing by standarized single disk method. *Am J Clin Pathol 45*: 493–496.

Chakravarty AK, Dastidar PPG, Pakrashi SS (1982): Studies on Indian medicinal plants. Part 67. Simple aromatic amines from *Justicia gendarrusa*. Carbon-13 NMR spectra of the bases and their analogs. *Tetrahedron 38*: 1797–1802.

Chariandy CM, Seafort CE, Phelps RH, Pollard GV, Khambay BP (1999): Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J Ethnopharmacol 64*: 265–270.

Chen CC, Hsin WC, Huang YL (1998): Six new diarylbutane lignans from *Justicia procumbens*. *J Nat Prod 61*: 227–229.

Chen CC, Hsin WC, Ko FN, Huang YL, Ou JC, Teng CM (1996): Antiplatelet arylnaphthalide lignans from *Justicia procumbens*. *J Nat Prod 59*: 1149–1150.

Day SH, Chiu NY, Won SJ, Lin CN (1999): Cytotoxic lignans of *Justicia ciliata*. *J Nat Prod 62*: 1056–1058.

de Smet PA (1985): A multidisciplinary overview of intoxicanting snuff rituals in the western hemisphere. *J Ethnopharmacol 13*: 3–49.

de Vries JX, Tauscher B, Wurzel G (1988): Constituents of *Justicia pectoralis* Jacq. 2. Gas chromatography/mass spectrometry of simple coumarins, 3-phenylpropionic acids and their hydroxy and methoxy derivatives. *Biomed Environ Mass Spectrom 15*: 413–417.

Domínguez XA, Achenbach H, González C, Ferré-D'Amare AR (1990): Estudio químico del "Muitle" (*Justicia spicigera*). *Rev Latinoam Quim 21*: 142–143.

Euler KL, Alam M (1982): Isolation of kaempferitrin from *Justicia spicigera*. *J Nat Prod 45*: 211–212.

Finney DJ (1971): *Probit Analysis*. Canada, Cambridge University Press, 3rd edition, pp. 135–145.

Fukamiya N, Lee KH (1986): Antitumor agents, 81. Justicidin-A and diphyllin, two cytotoxic principles from *Justicia procumbens*. *J Nat Prod 49*: 348–350.

Ghosal S, Banerjee S (1979): Synthesis of retrochinensis; a new naturally occurring 4-aryl-2,3-naphtalide lignan. *J Chem Soc Chem Commun 4*: 165–166.

Ghosal S, Banerjee S, Frahm AW (1979a): Chemical constituents of *Justicia*. III. Prostalidins A, B, C and retrochinensis: a new antidepressant; 4-aryl-2,3-naphtalide lignans from *Justicia prostrata*. *Chem Ind 23*: 854–855.

Ghosal S, Banerjee S, Jaiswal DK (1980): Chemical constituents of *Justicia*. Part 2. New furofurano lignans from *Justicia simplex*. *Phytochemistry 19*: 332–334.

Ghosal S, Banerjee S, Srivastava RS (1979b): Simplexolin, a new lignan from *Justicia simplex*. *Phytochemistry 18*: 503–505.

Ghosal S, Srivastava AK, Srivastava RS, Chattopadhyay S, Maitra M (1981): Chemical constituents of *Justicia*. Part 4. Justicisaponin-I, a new triterpenoid saponin from *Justicia simplex*. *Planta Med 42*: 279–283.

Heywood VH, Moore DM (1978): *Flowering plants of the world*. England, Oxford University Press, pp. 75–81.

Ibrahim B, M'batchi B, Mounzeo H, Bourobou BHP, Posso P (2000): Effect of Tephrosia *vogelii* and *Justicia extensa* on *Tilapia nilotica* in vivo. *J Ethnopharmacol 69*: 99–104.

Ismail LD, Lorenz P, Stermitz FR (1998): Isolation and synthesis of an alpha-malamic acid derivative from *Justicia ghiesbreghtiana*. *J Nat Prod 61*: 1174–1176.

Jawets E, Melnick JL, Adelberg EA (1976): *Review of medical microbiology*. California, Lange Medical Publications, 12th edition, pp. 230–238.

Joseph H, Gleye J, Moulis C, Mensah LJ, Roussakis C, Gratas C (1988): Justicidin B, a cytotoxic principle from *Justicia pectoralis*. *J Nat Prod 51*: 599–600.

Leal LK, Ferreira AA, Bezerra GA, Matos FJ, Viana GS (2000): Antinociceptive, antiinflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. *J Ethnopharmacol 70*: 151–159.

Lin J, Yu Z, Zhong M (1982): Antiarrhythmic principle of *Justicia procumbens*. *Yaoxue Tongbao 17*: 365–368.

Lorenz P, Stermitz FR, Ismail LD (1999): An amide of L-threo-gamma-hydroxyglutamic acid from *Justicia ghiesbreghtiana*. *Phytochemistry 52*: 63–66.

MacRae WD, Towers GH (1984): *Justicia pectoralis*: a study of the basis for its use as hallucinogenic snuff ingredient. *J Ethnopharmacol 12*: 93–111.

Mahabir D, Gulliford MC (1997): Use of medicinal plants for diabetes in Trinidad and Tobago. *Pan Am J Public Health 1*: 174–178.

Meurer-Grimes B, McBeth MC, Hallihan B, Delph S (1996): Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. *Int J Pharmacog 34*: 243–246.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982): Brine shrimp: A convenient general bioassay for active plants constituents. *Planta Med 59*: 250–251.

Mills J, Pascoe KO, Chambers J, Melville GN (1986): Preliminary investigations of the wound-healing properties of a Jamaican folk medicinal plant (*Justicia pectoralis*). *West Indian Med J 35*: 190–193.

Ohta K, Munakata K (1970): Justicidin C and D, the 1-methoxy-2,3-naphtalide lignans, isolated from *Justicia procumbens*. *Tetrahedron Lett 12*: 923–925.

Ojala T, Vuorela P, Kiviranta J, Vuorela H, Hiltunen R (1999): A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. *Planta Med 65*: 715–718.

Okigawa M, Maeda T, Kawano N (1970a): Isolation and structure of three new lignans from *Justicia procumbens* var. *leucantha*. *Tetrahedron 26*: 4301–4305.

Okigawa M, Maeda T, Kawano N (1970b): Isolation of neojusticin from *Justicia procumbens* L. *Chem Pharm Bull 18*: 862–863.

Olaniyi AA (1980): *Justicia flava* Vahl: studies on the petroleum ether extract of the leaves, stems and roots. *Niger J Pharm 11*: 133–134.

Olaniyi AA, Powell JW (1980): Lignans from *Justicia flava*. *J Nat Prod 43*: 482–486.

Ponce-Macotela M, Navarro-Alegría I, Martínez-Gordillo MN, Álvarez-Chacón R (1994): *In vitro* effect against *Giardia* of 14 plant extracts. *Rev Invest Clin 46*: 343–347.

Rajendiran C, Pai BR, Subramanian, PS (1991): Lignans of *Justicia glauca* Rottl. *Indian J Chem Sect B 30B*: 681–683.

Raju GV, Pillai KR (1989): Lignans from *Justicia tranquebariensis* Linn. F. *Indian J Chem Sect B 28B*: 558–561.

Rigby W, Hunt BJ (1967): Methods, apparatus: New product research, process development and design. Short column chromatography. *Chem Ind 4*: 1868–1869.

Sastry KV, Rao EV, Pelter A, Ward RS (1979): 4-Aryl-2,3-naphtalide lignans from *Justicia simplex*. *Indian J Chem Sect B 17B*: 415–416.

Schnee L (1984): *Plantas comunes de Venezuela*. Caracas, Venezuela. Ediciones de la biblioteca de la Universidad Central de Venezuela, 3era edición, pp. 413–415.

Subbaraju GV, Kumar KK, Raju BL, Pillai KR, Reddy MC (1991): Justiciresinol, a new furanoid lignan from *Justicia glauca*. *J Nat Prod 54*: 1639–1641.

Telefo PB, Moundipa PF, Tchana AN, Tchouanguep DC, Mbiapo FT (1998): Effects of an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera verticillata* on some physiological and biochemical parameters of reproduction in immature female rats. *J Ethnopharmacol 63*: 193–200.

Tiwari KP, Masood M, Rathore YK, Minocha PK (1978): Anthocyanins from the flowers of some medicinal plants. *Vijn Parish Anusan Pat 21*:177–178.

Trujillo JM, Jorge RE, Navarro E, Boada J (1990): Lignans from *Justicia hyssopifolia*. *Phytochemistry 29*: 2991–2993.

Wahi SP, Wahi AK, Kapoor R (1974): Chemical study of the leaf of *Justicia gendarrusa* Burm. *J Res Indian Med 9*: 65–66.

Wong W (1976): Some folk medicinal plants from Trinidad. *Econ Bot 30*: 103–142.