Phytomedicine, Vol. 6(3), pp. 187–195

http://www.urbanfischer.de/journals/phytomed

Phytomedicine

Antimicrobial, antitumor and antileishmania screening of Medicinal Plants from Guinea-Bissau

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Summary

Following an ethnobotanical search carried out in Guinea-Bissau, eighteen extracts derived from sixteen medicinal species were screened for antimicrobial, antitumor and antileishmania activity. Significant antitumor activity was found for *Holarrhena floribunda* against KB (squamous carcinoma), SK-Mel 28 (melanoma), A 549 (lung carcinoma) and MDA-MB 231 (mamma carcinoma) cell lines, with corresponding IC_{50} values of 7.9, 9.0, 3.4 and 9.9 µg/ml. *Khaya senegalensis* and *Anthostema senegalense* exhibited a significant activity against *Leishmania donovani* with IC_{50} values of 9.8 and 9.1 µg/ml, respectively. Most of the extracts showed week or moderate antibacterial and antifungal activity, with MIC values in the range 0.25–1.0 mg/ml. Active extracts were submitted to bioassay-guided fractionation, and the IC_{50} and MIC of the active fractions were determined.

Key words: Medicinal plants, Guinea-Bissau, antimicrobial activity, antitumor activity, antileishmania activity.

Introduction

The resort to plants as medicines, represents a primary health care measure of the native population of Guinea-Bissau. Nevertheless, in spite of the existing abundant literature on African herbal *materia medica* (Bever, 1986; Iwu, 1993; Hostettmann et al., 1996; OUA, 1985), only a few medicinal plants from Guinea-Bissau have been the subject of ethnobotanical (Santo, 1948; Vieira, 1959; Gomes and Diniz, 1993), pharmacological (Prista and Alves, 1958; Silva et al., 1996; Silva et al., 1997; Silva, et al., 1997; Silva et al., 1964) and phytochemical investigation (Prista et al., 1962; Ferreira et al., 1963; Ferreira et al., 1963a; Ferreira et al., 1963b; Ferreira et al., 1965; Prista et al., 1965; Silva et al., 1963; Ferreira et al., 1968; Abreu and Noronha, 1997; Abreu and Pereira, 1998; Abreu et al., 1998; Paulo, et al., 1995).

In the course of an ethnomedical survey carried out in the Contuboel region of Guinea-Bissau, several medicinal species were collected, and the local therapeutic uses were registered in accordance with the depositions of the Fulani and Mandinga traditional healers (Table 1). As part of a program oriented towards the discovery of bioactive natural products, we screened eighteen extracts from sixteen plants for antimicrobial, antitumor and antileishmania activity. The activity of the extracts demonstrating a positive response in any of the tested assays, was enriched by bioassay-guided fractionation for further isolation of the active principles.

0944-7113/99/06/03-187 $ 12.00/0

Materials and Methods

Plant material

Plants were collected in the Contuboel region, Guinea-Bissau, in December 1994, with the exception of *Khaya senegalensis* and *Anthostema senegalense*, which were collected in February 1991. Identification of plant material was authenticated at the Herbarium of the Centro de Botânica, Instituto de Investigação Científica Tropical (LISC), Lisbon, where voucher specimens are preserved.

Extraction and fractionation

Air dried plant material was powdered and extracted in a Soxhlet apparatus with MeOH, EtOH or MeOH/H_2O (Table 1), and the extracts were submitted to reversed-phase HPLC analysis and fractionation. Analytical HPLC was performed on a D-7000 Merck instrument equipped with a Lichrospher 100 RP-18 column (250 mm × 4 mm, 10 µm) and a DAD-detector in a range 200–450 nm. The mobile phase was a linear gradient system of MeOH-H_2O (0:10 to 10:0 in 30 min), flow rate 0.8 ml/min. Preparative HPLC fractionation was conducted at 226 nm with the same instrument and a Lichrospher 100 RP-18 column (250 mm × 8 mm, 10 µm). The mobile phase was a linear gradient system of MeOH-H_2O (0:10 to 10:0 in 30 min), flow rate 3.2 ml/min, injection volume 330 µl (50 mg of extract). Ten fractions were collected with a fraction interval of 3 min starting from 3 to 33 min. Organic solvent was removed from all fractions by TURBOVap evaporation for four hours. The water residues were freeze dried over night and each fraction was dissolved in 250 µl DMSO. A stock solution (5 mg/ml DMSO) and the fractionated extracts were linear diluted to obtain final concentrations of 1.0, 0.5, 0.25, 0.125, 0.063, 0.032, 0.016, 0.08, 0.04 and 0.02 mg/ml. *Anthostema senegalense* extract was chromatographed on Sephadex LH-20 (40 cm × 5 cm) with gradient system of H_2O-MeOH (100:0 to 30:70), affording 10 crude subfractions. Following qualitative TLC analysis on silica gel (EtOAc-H_2O-HCO_2H, 18:1:1) appropriate fractions (15 ml) were combined and evaporated.

Evaluation of antibacterial and antifungal activity

The extracts were tested against the Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* DSM 2026, *Citrobacter freundii* Biotecon 1219, the Gram positive bacteria *Staphylococcus aureus* ATCC 20232, *Streptococcus pyogenes* DSM 2071, *Bacillus subtilis* ATCC 6051, *Listeria monocytogenes* DSM 20600, and fungi *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404.

Qualitative antimicrobial activity of the extracts was evaluated using the agar plate diffusion test (Van den Berghe and Vlietinck, 1991; DIN 58940, 1994). Standard Mueller-Hinton agar was used as medium for bacteria, and Sabouraud agar was used for fungi cultivation. The extract was dissolved in sterile DMSO at concentrations of 1 and 5 mg/ml, diluted in agar Mueller-Hinton medium, and the plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi, 5% CO_2. The diameters of the resulting inhibition zones were measured and compared to Penicillin G and Nystatin as reference controls. All assays were done in duplicate.

The minimum inhibitory concentration (MIC) was determined for the active extracts, against *P. aeruginosa, K. pneumoniae, C. freundii, S. aureus* and *L. monocytogenes*, in the Mueller-Hinton-Bouillon medium using a test sample concentration ranging from 0.02 to 1.0 mg/ml. HPLC fractions of selected extracts were screened as well against and *L. monocytogenes, S. aureus, S. pyogenes* and *C. albicans*, and the diameters of the corresponding inhibition zones were measured.

Evaluation of the antitumor activity

Antitumor activity was determined according to the NCI standards, against the following human tumor cell lines: squamous carcinoma (KB), melanoma (SK-Mel 28), lung carcinoma (A 549), and mamma carcinoma (MDA-MB 231). IC_{50} values were determined for raw extracts, as well as for chromatographic fractions which inhibited the growth of tumor cells at least by 80%, compared to control. Cell suspensions were diluted according to the particular cell type and the expected cell density (typically 5,000–10,000 cells per well based on cell growth characteristics), and added (100 µl) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37 °C for stabilisation and adherence. Test compounds were dissolved in DMSO, and the IC_{50} values evaluated at concentrations of 20.0, 2.0 and 0.2 µg/ml. Incubations lasted for 72 h in 5% CO_2 atmosphere and 100% humidity. Cell proliferation was quantified by the sulforhodamine B assay. A plate reader was used to read the optical densities at 490 nm. Cis-Platin was used as standard antiproliferative agent.

Evaluation of antiprotozoal activity

Leishmania donovani LV9 (Channon et al., 1984), *L. infantum*, strain D.SCH., isolated in 1995 at the Bernhard Nocht-Institut, Hamburg, Germany from a case of infant VL (Mauël et al., 1975), *L. enriettii* (Mauël et al., 1975), and *L. major* LV39 (Müller et al., 1997), were maintained by animal passage (except *L. infantum*) and cryopreserved in liquid nitrogen. Promastigotes were

Table 1. Collected plants and traditional uses.

Family, species	Part of plant	Extract	Local ethnobotanical information
ANACARDIACEAE			
Ozoroa insignis Del. subsp. *latifolia* (Engl.) R. Fern. var. *intermedia* R. Fern.	Roots	MeOH	Infusion is taken by women after childbirth to increase lactation
APOCYNACEAE			
Holarrhena floribunda (G. Don) T. Durand & Schinz Synonyms: *H. africana* A. DC.; *H. wulfsbergii* Stapf; *Rondeletia floribunda* G. Don	Stem	EtOH	Antidote against poisonous snake bites
CAESALPINIACEAE			
Daniellia oliveri (Rolfe) Hutch. & Dalz. Synonyms: *Paradaniellia oliveri* Rolfe; *D. thurifera sensu* Oliv. non Benn.	Bark	MeOH	Decoction is used against migraine, chronic headache and fevers
Piliostigma thonningii (Schumach.) Milne-Redh. Synonyms: *Bauhinia thonningii* Schumach.	Bark	MeOH	Used as hemostatic in the treatment of wounds and ulcers
COMBRETACEAE			
Combretum micranthum G. Don	Leaves	EtOH	Infusion is used for the treatment of colic, nausea and cough
Synonyms: *C. altum* Perr. ex DC.; *C. floribundum* Engl.& Diels; *C. raimbaultii* Heckel *Combretum collinum* Fresen.	Roots	MeOH	Decoction is used to relieve toothache
EUPHORBIACEAE			
Anthostema senegalense A. Jus.	Leaves	MeOH	Infusion is used as anti-inflammatory
LORANTHACEAE			
Tapinanthus bangwensis (Engl. & K. Krause) Danser Synonyms: *Loranthus bangwensis* Engl. & K. Krause	Stem	EtOH	Decoction of entire plant is used urinary incontinence
MELIACEAE			
Khaya senegalensis (Desr.) A. Jus. Synonyms: *Swietenia senegalensis* Desr.	Bark	MeOH	The infusion is used to relief "body pain" and in veterinary, as parasiticide
MIMOSACEAE			
Parkia biglobosa (Jacq.) Benth.	Bark	MeOH	Recommended as astringent, infusion used against dental caries
Dichrostachys cinerea (L.) Wight & Arn. subsp. *platycarpa* (Welw. ex W. Bull) Brenan & Brummitt var. *platycarpa* Synonyms: *D. glomerata* (Forck.) shiov.; *D. platycarpa* W. Bull	Bark	MeOH	Used as hemostatic in the treatment of wounds. Treatment of rheumatism ("bone diseases")
PAPILIONACEAE			
Detarium microcarpum Gill. & Perr.	Bark	EtOH	Decoction is used to treat anemia
Pterocarpus erinaceus Poir.	Bark	MeOH	Infusion is used to treat anemia and gonorrhoea
RUBIACEAE			
Morinda geminata DC.	Leaves	MeOH/H_2O	Infusion is used as purgative by women after childbirth
Sarcocephalus latifolius (Sm.) Bruce	Roots	EtOH	The decoction of roots and bark is used in the treatment of gastrointestinal troubles, and the decoction of the leaves is administered internally and as wash and liniment in the treatment of fever. The decoction of the roots is also given in the treatment of venereal diseases, while the bark is used to wash wounds and as odontalgic remedy.
Synonyms: *Nauclea lalifolia* Sm.; *N. esculenta* (Sabine) Merr.; *S. esculentus* Sabine; *S. russeggeri* Kotschy ex Schweinf.	Stem bark	EtOH	
	Leaves	EtOH	
ZINGIBERACEAE			
Aframomum alboviolaceum (Ridley) K. Schum.	Rhizomes	BuOH	Used as diuretic and anthelmintic, and in veterinary, as parasiticide.

cultured in GM at 25 °C, 5% CO_2 in a humidified incubator. The parasites were passaged every 3–4 days. The effects of different plant extracts on the growth of *Leishmania* promastigotes was assessed by monitoring the MTT metabolism (Mosmann, 1983). A parasite concentration of 2×10^5/ml (1×10^4/100 µl in each well) from a 4-day-old culture was used in the test. Parasites were incubated on a microtiter plate (96 wells) in the presence of different concentrations of the extracts, Pentostam as reference agent, or RS medium alone. The stock solution was diluted with RS medium to achieve final well concentrations of 50.0, 25.0, 12.5, 6.25 and 3.12 µg/ml for the crude extracts. A final concentration of DMSO below 1.0% did not affect the parasite

Table 2. Antimicrobial activity of plant extracts.

	Microorganisms*																			
	1		2		3		4		5		6		7		8		9		10	
	Inhibition zones (mm) at extract concentration of 1 mg/ml (A) and 5 mg/ml (B)																			
Plant	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Detarium microcarpum	0	0	12	10	10	0	11	0	12	12	9	12	13	13	0	0	0	11	0	0
Parkia biglobosa	0	0	12	10	0	0	10	0	11	13	8	11	8	9	0	0	0	13	0	0
Pterocarpus erinaceus	0	0	10	10	0	0	0	0	10	13	8	11	3	10	0	0	0	15	0	0
Tapinanthus bangwensis	0	0	10	10	0	0	0	0	11	13	8	10	10	9	0	0	0	12	0	0
Aframomum alboviolaceum	0	0	10	9	0	0	0	0	0	11	8	10	0	10	0	0	0	9	0	0
Khaya senegalensis	0	0	10	9	0	0	0	0	9	10	8	0	0	0	0	0	0	9	0	0
Anthostema senegalense	0	0	10	8	0	0	0	0	0	0	8	13	0	10	0	0	0	10	0	0
Daniellia oliveri	0	0	10	9	0	0	0	0	10	11	8	0	0	0	0	0	0	10	0	0
Morinda geminata	0	5	9	0	0	0	0	0	0	0	0	8	10	11	0	0	0	0	0	0
Ozoroa insignis	0	0	10	11	0	0	0	7	9	9	9	13	0	9	8	0	0	11	0	0
Piliostigma thonningii	0	0	9	11	0	0	0	8	10	11	0	8	9	12	0	0	0	12	0	0
Combrerum micranthum	0	0	9	8	0	0	0	0	10	10	0	8	0	8	0	0	0	11	0	0
Combretum collinum	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	14
Holarrhena floribunda	0	0	9	9	0	0	0	0	0	12	0	0	0	0	0	0	0	18	0	0
Dichrostachys cinerea	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sarcocephalus latifolius, roots	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
Sarcocephalus latifolius, stem bark	0	0	9	9	0	0	0	0	0	8	0	0	8	8	0	0	0	10	0	0
Sarcocephalus latifolius, leaves	0	0	8	0	0	0	0	0	8	0	0	0	0	0	0	0	0	8	0	0
Penicillin G	0	0	0	0	0	0	0	0	37	37	40	40	31	31	22	22	–	–	–	–
Nystatin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14	14	0	0

* 1. *Escherichia coli*; 2. *Pseudomonas aeruginosa*; 3. *Klebsiella pneumoniae*; 4. *Citrobacter freundii*; 5. *Staphylococcus aureus*; 6. *Streptococcus pyogenes*; 7. *Listeria monocytogenes*; 8. *Bacillus subtilis*; 9. *Candida albicans*; 10. *Aspergillus niger*

growth. Each concentration was tested in duplicate. The plates were incubated at 25 °C for 72 h in 5% CO_2. MTT (20 µl/well) was then added for another 4 h. MTT processing was stopped and formazan crystals solubilized by adding 50 µl SDS (20%) and incubating overnight at 37 °C. The relative amount of formazan produced by viable cells was measured photometrically at 570 nm by blanking against an appropriate control.

Results and Discussion

The antibacterial spectra and the minimum inhibitory concentrations of extracts are displayed in Table 2. Crude extracts were found mostly to be moderate or not active against tested bacteria with the exception of *Detarium microcarpum*, *Parkia biglobosa*, *Pterocarpus erinaceus* and *Tapinanthus bangwensis*, when tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The remaining extracts didn't showed significant activity against the tested microorganisms, with the exception of *P. aeruginosa*, which was the most sensitive strain (all the extracts exhibited inhibition zones at both concentrations), followed by *S. aureus* (10 extracts with inhibition zones at *c* 1 mg/ml and 11 extracts with inhibition zones at *c* 5 mg/ml). From the two tested fungi, *Candida albicans* was sensitive to fifteen extracts at a concentration of 5 mg/ml.

The minimum inhibitory concentration (MIC) of twelve extracts was determined for susceptible bacteria according to the previous qualitative evaluation. The results indicated moderate activity for most of the extracts with MIC values ranging from 0.25 to 1.0 mg/ml (Table 3). *D. microcarpum*, *P. biglobosa* and *T. bangwensis* extracts were further submitted to reversed-phase HPLC, and an enrichment of activities against *L. monocytogenes*, *S. aureus*, *S. pyogenes* and *C. albicans*,

Table 3. Minimum inhibitory concentration (MIC) for selected extracts and microorganisms.

Plant	Microorganism	MIC (μg/ml)
Detarium microcarpum	*Pseudomonas aeruginosa*	0.5
	Klebsiella pneumoniae	1.0
	Citrobacter freundii	0.5
	Staphylococcus aureus	0.25
	Listeria monocytogenes	1.0
Parkia biglobosa	*Pseudomonas aeruginosa*	1.0
	Citrobacter freundii	0.5
	Staphylococcus aureus	0.25
Pterocarpus erinaceus	*Pseudomonas aeruginosa*	1.0
	Staphylococcus aureus	0.25
Tapinanthus bangwensis	*Pseudomonas aeruginosa*	1.0
	Staphylococcus aureus	0.25
	Listeria monocytogenes	1.0
Aframomum alboviolaceum	*Pseudomonas aeruginosa*	1.0
Khaya senegalensis	*Pseudomonas aeruginosa*	1.0
Anthostema senegalense	*Pseudomonas aeruginosa*	1.0
Daniellia oliveri	*Pseudomonas aeruginosa*	1.0
	Staphylococcus aureus	0.25
Morinda geminata	*Listeria monocytogenes*	1.0
Ozoroa insignis	*Pseudomonas aeruginosa*	1.0
Piliostigma thonningii	*Staphylococcus aureus*	1.0
Combretum micranthum	*Staphylococcus aureus*	0.5

Table 4. Antimicrobial activity of HPLC fractions from selected extracts.

	Inhibition zones (mm) at fraction concentration of 1 mg/ml				
		Microorganisms			
Plant	Fraction*	*L. monocytogenes*	*S. aureus*	*S. pyogenes*	*C. albicans*
Detarium microcarpum	4	11	13	15	14
	5	19	13	14	13
Parkia biglobosa	4	13	21	19	13
	5	9	10	18	9
Tapinanthus bangwensis	4	14	16	20	12
	5	10	12	12	8
Penicillin G		31	37	40	–
Nystatin		–	–	–	14

* order of elution in HPLC run

could be observed in some chromatographic fractions (Table 4).

Regarding *T. bangwensis, D. microcarpum, Pterocarpus erinaceus, Ozoroa insignis, Aframomum alboviolaceum, Anthostema senegalense, Morinda geminata* and *Daniellia oliveri,* as far as we know, this is the first reported *in vitro* antimicrobial evaluation of these plants.

Concerning *P. biglobosa,* these results did not reproduced those encountered in a previous antimicrobial screening of savanna plants (Adoum et al., 1997), in which the ethanolic extract of *P. biglobosa* stembark was found to be inactive against *C. albicans* and weakly active against a panel of Gram-negative and Gram-positive bacteria. On the other hand, in respect to the remaining species, we didn't confirmed the antimicrobial activity which has been previously reported for *Combretum micranthum* leaves and stembark (Mela, 1950; Malcolm and Sofowora, 1969; Laurens et al. 1985; Adoum et al., 1997; Ferrea et al., 1993), *Piliostigma thonningii* leaves (Ibewuike et al., 1997), *Khaya senegalensis* stems and stembark (Malcolm and Sofowora, 1969; Adoum et al., 1997), *Holarrhena floribunda* bark and stembark (Hoyer et al., 1978; Chukwurah, 1997), *Combretum collinum* bark (Almagboui et al., 1988), *Dichrostachys cinerea* fruits (Almagboui et al., 1988), and *Nauclea latifolia* roots (Deeni and Hussain 1991).

Table 5. Antitumor activitv of plant extracts.

	IC_{50} (μg/ml)			
Plant	KB	SK-MEL 28	A-549	MDA-MB 231
Detarium microcarpum	*	*	*	14.8
Parkia biglobosa	*	*	27.3	13.5
Pterocarpus erinaceus	*	*	*	*
Tapinanthus bangwensis	*	*	*	*
Aframomum alboviolaceum	23.2	*	*	*
Daniellia oliveri	*	*	*	23.7
Ozoroa insignis	30.5	*	22.0	15.4
Piliostigma thonningii	*	*	*	*
Combretum collinum	*	*	*	*
Holarrhena floribunda	7.9	9.0	3.4	9.9
Sarcocephalus latifolius, roots	*	*	*	21.1
Sarcocephalus latifolius, bark	*	*	*	*
Sarcocephalus latifolius, leaves	19.7	32.8	29.9	*

* IC_{50} > 20 μg/ml

Table 6. Antitumor activity of HPLC extract fractions.

		% gowth of tumor cells			
Plant	Fraction*	KB	SK-MEL 28	A-549	MDA-MB 231
Detarium microcarpum	4	44.6	38.3	19.7	35.6
	5	25.5	77.7	32.3	27.8
Parkia biglobosa	3	100	29.9	25.7	34.4
	4	20.1	44.1	16.8	27.8
	5	31.6	77.4	19.0	30.8
Tapinanthtus bangwensis	4	13.8	41.1	18.8	33.2
	5	28.4	75.5	67.2	23.5
Aframomum alboviolaceum	4	30.1	37.0	16.2	20.5
	5	45.6	42.0	26.1	33.6
Khaya senegalensis	4	15.8	46.1	19.2	26.8
Anthostema senegalense	4	76.2	42.0	20.2	20.3
	5	65.5	26.7	15.2	19.7
	9	41.9	27.1	48.3	86.2
Daniellia oliveri	4	100	84.2	100	29.5
Piliostigma thonningii	4	16.6	38.4	51.5	72.5
Combretum micranthum	4	100	95.0	81.8	48.8
Combretum collinum	4	64.5	94.6	87.4	95.8
Holarrhena floribunda	4	74.3	77.4	77.3	83.8
	6	80.3	90.5	74.9	91.7
	7	4.7	8.1	7.9	7.3
	8	8.0	18.2	13.3	25.3

* order of elution in HPLC run

From the eighteen plant extracts screened for cytotoxicity, *H. floribunda* exhibited the most significant activity in the four tested cell lines, with IC_{50} values ranging from 3.4 to 9.9 μg/ml (Table 5). Despite the extensive phytochemical and pharmacological investigation of *H. floribunda* (Bever, 1986; Iwu, 1993), there are no ethnomedical records on the use of this species in "cancer" medicine, and none of its known constituents (alkaloids, triterpenes, phenolic acids, flavonoids) are cytotoxic. The exception is progesterone, previously isolated from the leaves (Leboeuf et al., 1969), which is reported to possess mammary-carcinoma inhibiting potential (Michna et al., 1995). Nevertheless, this steroid was not present in the stem bark extract. Following HPLC fractionation, fractions 7 and 8 (Table 6) inhibited the growth of tumor cells by 74.7% (fraction 8,

Table 7. Leishmanicidal activity of *Anthostema senegalensis* Sephadex LH-20 fractions.

	IC_{50} values (µg/ml)			
Fraction*	*Leishmania donovani*	*Leishmania major*	*Leishmania infantum*	*Leishmania enriettii*
Extract	9.8	not tested	not tested	not tested
1	>25	>25	7.9	>25
2	>25	>25	11.9	>25
3	>25	>25	>25	>25
4	>25	10.8	7.9	10.8
5	1.67	1.9	0.2	1.4
6	2.66	>25	>25	3.3
7	>25	>25	>25	>25
8	>25	>25	>25	>25
9	>25	>25	>25	>25
10	2.5	4.1	1.3	1.9

* order of elution in column chromatography

MDA-MB cells) and 95.3% (fraction 7, KB cells). The isolation and identification of the corresponding active principles is in progress.

The root extract of *O. insignis* showed moderate activity in KB, A 549 and MDA-MB cell lines, with IC_{50} values of 30.5, 22.0 and 15.5 µg/ml, respectively, but this activity was not found in the HPLC fractions. Other chromatographic supports and eluents should be experimented in the fractionation step, in order to isolate the active principles. Although several traditional uses of the roots of *O. insignis* have been reported (Burkill, 1985), so far, only stembark and stemwood were investigated, for *in vitro* topoisomerase inhibition (Wall et al., 1996).

The extract of *S. latifolius* leaves, was active against KB, SK-MEL and A 549 cell lines, with IC_{50} values of 19.7, 32.8 and 29.9 µg/ml, respectively, whereas the root extract only inhibited the growth of MDA-MB tumor cells (IC_{50} 21.1 µg/ml), and the bark extract was inactive in all the tested systems. The anticancer activity against transplantable sarcoma 180 tumors and against Lewis lung carcinoma, of the leaf extract of *S. latifolius*, has been previously reported (Abbot et al., 1966), but the nature of the active agents remains unknown.

P. biglobosa extract showed moderate activity in two cell lines (A 549 and MDA-MB), whereas the corresponding HPLC fractions inhibited the growth of tumor cells by 70.1% (fraction 3, SK-MEL cells) and 83.2% (fraction 4, A-549). This are the first published data of antitumor activity in *P. biglobosa* extracts, although moderate activity of the ethanolic bark extract against *Artemia salina* (LD_{50} 985.3 µg/ml) has been reported (Spatafora and Tringali, 1996), and antiplatelet activity was found in seeds (Rendu et al., 1993).

D. microcarpum and *D. oliveri* extracts showed moderate activity in MDA-MB cell system, with IC_{50} values of 14.8 and 23.7 µg/ml, respectively, whereas *A. alboviolaceum* was active against KB cells (IC_{50} 23.2 µg/ml). This last result is in accordance with the reported cytotoxicity of *Aframomum spp.* extracts, which is attributed to aframodial and related diterpenoids (Ayafor et al., 1994a, 1994b). Antitumor activity of *D. microcarpum* stembark extracts has been previously predicted on the basis of the brine shrimp assay (Fatope et al., 1993), but no further investigation has yet been done on the corresponding cytotoxicity. Several catechins with anti-HIV-1 activity have been isolated from a methanolic bark extract (Aquino et al., 1995). According to previous pharmacological studies on *D. oliveri*, analgesic, antypiretic and antiinflammatory activities were attributed to stembark extracts (Onwukaeme, 1995).

In some cases, moderate activity against one or two cell lines was found in HPLC fractions from inactive extracts. This was the case for fraction 4 of *T. bangwensis*, *K. senegalensis* and *P. thonningii*, and fraction 5 of *A. senegalense* (Table 5). To our knowledge, there are no published data referring antitumor activity in these four species.

Significant leishmanicidal activity against *Leishmania donovani* was found for *K. senegalensis* and *A. senegalense* extracts, with IC_{50} values of 9.8 and 9.1 µg/ml, respectively. All other extracts were inactive (IC_{50} > 25 µg/ml).

K. senegalensis has been the subject of extensive phytochemical and pharmacological investigation (Bever, 1986; Iwu, 1993; Olayinka et al., 1994), mainly for its antimalarial properties, which are probably attributed to gedunin and other limonoids. The extracts of 22 species of Meliaceae and gedunin derivatives were recently examined for antimalarial activity against *Plasmodium falciparum* (McKinnon et al., 1997), but to our knowledge, this is the first reported antileishmania activity in this plant.

Concerning *A. senegalense,* there are no bibliographic records (CA, CAPLUS, NAPRALERT, MEDLINE) on the use of this plant in traditional medicine or any related phytochemical studies. Following Sephadex LH-20 fractionation of the crude extract, several fractions showed significant activity against *Leishmania donovani, L. major, L. infantum* and *L. enriettii* (Table 7), which are leads for the isolation of leishmanicidal agents.

Our present results will be the basis for bioassay-guided isolation of the antimicrobial, antitumor and antileishmania compounds from the active plant extracts.

Acknowledgements

We wish to thank Prof. Maurer, Institut für Pharmazie I, Freie Universität Berlin, for providing tumor cell lines, and Prof. E. Gomes, Faculty of Pharmacy of Lisbon, for the gift of *Khaya senegalensis* and *Anthostema senegalense* samples.

We would also like to thank Umaru Candé for its helpful assistance in plant collection.

This work has been supported by PRAXIS XXI, under research contract PRAXIS/PSAU/P/SAU/103/96.

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