Bioactive Chemical Constituents and Comparative Antimicrobial Activity of Callus Culture and Adult Plant Extracts from *Alternanthera tenella*

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Crude extracts of a callus culture (two culture media) and adult plants (two collections) from *Alternanthera tenella* Colla (Amaranthaceae) were evaluated for their antibacterial and antifungal activity, in order to investigate the maintenance of antimicrobial activity of the extracts obtained from plants *in vivo* and *in vitro*. The antibacterial and antifungal activity was determined against thirty strains of microorganisms including Gram-positive and Gram-negative bacteria, yeasts and dermatophytes. Ethanolic and hexanic extracts of adult plants collected during the same period of the years 1997 and 2002 [Ribeirão Preto (SP), collections 1 and 2] and obtained from plant cell callus culture in two different hormonal media (AtT43 and AtT11) inhibited the growth of bacteria, yeasts and dermatophytes with inhibition halos between 6 and 20 mm. For the crude extracts of adult plants bioassay-guided fractionation, purification, and isolation were performed by chromatographic methods, and the structures of the isolated compounds were established by analysis of chemical and spectral evidences (UV, IR, NMR and ES-MS). Steroids, saponins and flavonoids (aglycones and C-glycosides) were isolated. The minimum inhibitory concentration (MIC) of the isolated compounds varied from 50 to 500 μg/mL.

Key words: Alternanthera tenella, Amaranthaceae, Callus Culture, Antimicrobial Activity

Introduction

Human infections, particularly those involving mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries. Dermatophytes, *Candida* sp., and bacteria are the most frequent pathogens. Several antimycotic drugs are available nowadays, but their use is limited by factors such as low potency, poor solubility, emergence of resistant strains, and drug toxicity. Therefore, the search for new, more effective antimicrobial agents is necessary and stimulates the research on new chemotherapeutic agents in medicinal plants (Penna *et al*., 2001; Quiroga *et al*., 2001; Cohen, 1992).

The family Amaranthaceae comprises many species with biological activities, which are used in nutrition and in traditional folk medicine (Tundis *et al*., 2008; Cai *et al*., 2003; Salvador *et al*., 2002; Siqueira, 1987). This family includes approximately 65 genera and 1000 species, and many plants of the Gomphreneae tribe have shown antimicrobial activity, such as *Alternanthera maritima* (Salvador *et al*., 2003, 2004), *Blutaparon portulacoides* (Salvador *et al*., 2002), *Gomphrena agrestis* (Ferreira *et al*., 2004), *G. martiana* and *G. boliviana* (Pomilio *et al*., 1992). The genus *Alternanthera* Forkssal includes 80 species and approximately 30 occur in Brazil (Siqueira, 1994/1995). Many species of *Alternanthera* are used for the treatment of infec-

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tions, as analgesic, antinociceptive, antiviral and diurectic. *Alternanthera tenella*, a herbaceous plant is frequently found in Brazil. In folk medicine, *A. tenella* has been used for the treatment of infections and as diuretic (Salvador *et al.*, 2006; Moraes *et al.*, 1994; Siqueira and Guimarães, 1984). Studies reported antioxidant, anti-inflammatory and immunomodulatory properties of *A. tenella* extracts (Biela *et al.*, 2008; Salvador *et al.*, 2006). No biotechnological and antimicrobial investigation has previously been reported.

Plant cell cultures, nowadays, are an important strategy for bioprospection of natural products. The *in vitro* large-scale production of bioactive compounds or extracts used as phytotherapeutics, pharmaceutical products, food additives, and cosmetics should be encouraged because of their scientific, economical or ecological importance (Wongwicha *et al.*, 2008; Avancini *et al.*, 2003; Bourgaud *et al.*, 2001; Fu *et al.*, 1999; Bouque *et al.*, 1998; Ames and Worden, 1997; Alfermann and Petersen, 1995). However, the *in vitro* production of bioactive metabolites or extracts can be regarded as the result of environmental conditions and the genotype of the cultured plant cells. Thus, factors like culture medium and its constituents (for example, carbohydrates, minerals, vitamins, phytohormones), light and temperature control the metabolism, the growth, the induction of calli or obtainment of suspension cultures and the differentiation. Callus cultures consist of undifferentiated plant cells and can be induced employing different hormonal combinations and other environmental conditions as already described (Gamborg and Phillips, 1995).

There is no report on biological activities of *A. tenella* against microorganisms in the literature; however, in a preliminary evaluation the crude extracts of adult plants have shown good antimicrobial activity. Therefore, the present work reports the results of a comparative study on antibacterial and antifungal activities of a callus culture and adult plant extracts from *A. tenella*, in order to investigate the maintenance of antimicrobial activity in extracts from plants obtained *in vivo* and *in vitro*. Moreover, the isolation and structure identification of the major antimicrobial constituents of the active extracts of *A. tenella* adult plants were performed in a bioassay-guided study.

Material and Methods

General experimental procedures

The ^{1}H, ^{13}C and 2D NMR spectra were recorded in $CDCl_3$, DMSO-d_6 and pyridine-d_5, with TMS as internal standard, on a Bruker Avance DRX spectrometer operating at 400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C NMR. ESI-MS was performed using an MS system – Quattro LC triple-stage quadrupole (Micromass, Manchester, UK) – fitted with a Z-electrospray interface operating in the positive (30 V) and negative (30 V) ion mode. IR spectra were obtained with KBr pellets using a Perkin Elmer model 1420 spectrophotometer, and UV spectra were recorded on a Hitachi U-3501 spectrophotometer. HPLC separations were carried out on a LC-6A Shimadzu liquid chromatograph equipped with a 3501 UV detector operating at 280 nm and using a reverse-phase separation procedure. Two Shim-pack ODS (C-18, 4.6 × 250 mm, and C-18, 20 × 250 mm) columns were used for analytical and preparative procedures. Reagents, culture medium and solvents were purchased from Difco Laboratories (Detroit, MN, USA), Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA).

Plant material

Whole plants of *Alternanthera tenella* Colla (Amaranthaceae) were collected at Alto da Boa Vista, Ribeirão Preto, SP, Brazil, in May 1997 (collection 1, collector number DAD0031) and May 2002 (collection 2, collector number DAD0055) and identified by Prof. Dr. Josafá Carlos de Siqueira (Pontifícia Universidade Católica, Rio de Janeiro, RJ, Brazil). A voucher specimen is deposited at the herbarium of the Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, SP, Brazil (register number SPFR 02968).

Callus induction and maintenance

Leaves from plantlets of *A. tenella* were washed in fresh tap water for 24 h, treated with 1% Benomil (fungicide-antiseptic, Sigma) for 40 min, and sterilized by stirring in 0.5% sodium hypochlorite solution for 20 min, followed by three successive rinses in sterile distilled water. Leaf explants were inoculated in Murashige and Skoog (1962) (MS) basal medium supplemented with 30.0 g/L sucrose (Aldrich), 1% (w/v) agar (Sigma),

and two different hormonal combinations: AtT43 [1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D, Sigma) and 1.0 mg/L kinetin (Kin, Sigma)] and AtT11 [2.5 mg/L α-naphthaleneacetic acid (NAA, Sigma) and 1.0 mg/L 6-benzyl-aminopurine (BAP, Sigma)]. The callus cultures were maintained on solid MS medium, subcultured every 30 d at (28 ± 2) ºC with a daily photoperiod of 16 h, harvested at day 30 of cultivation, and dried at 60 ºC.

Extraction and isolation of compounds

The air-dried, powdered whole *A. tenella* adult plants collected during the same period of the years 1997 and 2002 (collections 1 and 2) were extracted exhaustively by maceration at room temperature with hexane and ethanol successively in the proportion powder of plant:solvent = 1:2 (w/v). The spent biomass was filtered from the extracts and the solvents were removed under vacuum in a rotatory evaporator (below 40 ºC) to obtain the hexanic (Atpth) and ethanolic (Atpte) crude extracts of the adult plants. The aqueous lyophilized extracts (aerial parts and roots) were prepared in hot (FAHL, below 50 ºC, 30 min of extraction) and cold (FACL, at room temperature, 12 h of extraction) water.

Dry tissue obtained from the two different hormonal combination media (AtT43 and AtT11) was powered and successively extracted, in the proportion powdered callus culture:solvent = 1:2 (w/v), with hexane, chloroform, and ethanol at room temperature, overnight. Before extraction, the hexane, chloroform and ethanol extracts were grouped and the solvent evaporated under vacuum in a rotatory evaporator (below 40 ºC), to obtain the organic extracts (AtT11 and AtT43) of each callus culture medium. The antimicrobial activity was monitored in each stage of the isolation process.

The crude hexane extract (10 g) was fractionated by VLC (Kieselgel 60H, 1000 g) and eluted with a gradient of hexane, EtOAc and MeOH. The ethanolic crude extract (50 g) was suspended in methanol/water (9:1, v/v) and partitioned with hexane (yield 6 g) and dichloromethane (yield 4 g). The hydroalcoholic phase was submitted to an amberlite XAD-2 column eluted with water and ethanol. The ethanolic fraction (8 g) was suspended in methanol/water (1:4) and successively partitioned with *n*-butanol. The butanolic portion (4 g) was chromatographed over a 100 × 5 cm Sephadex LH-20 column using MeOH as eluent with a flow rate of 0.5 mL/min, yielding 110 fractions (10 mL each). The fractions obtained were monitored by TLC (silica gel plates, using the following solvent systems: *n*-BuOH/AcOH/H_2O, 65:15:25, upper phase; $CHCl_3$/MeOH/H_2O, 70:30:3) similarity and then purified by chromatography (columns containing Sephadex LH-20, polyvinylpyrrolidone or C-18 and CLAE in an analytical and preparative scale), recrystallization and precipitation. This procedure yielded the following flavone C-glycosides (Salvador *et al.*, 2006): vitexin (**13**), 2″-*O*-α-L-rhamnopyranosyl vitexin (**14**), 2″-*O*-β-D-glucopyranosyl vitexin (**15**) and acacetin 8-*c*-[α-L-rhamnopyranoyl-(1→2)-β-D-glucopyranoside] (**16**), as well as other flavonoids including kaempferol (**10**), quercetin 3-methyl ether (**11**) and quercetin (**12**).

Some compounds were also isolated from the hexane and dichloromethane phases: the Δ^5 steroids stigmasterol (**1**), β-sitosterol (**2**), campesterol (**3**); the Δ^7 steroids spinasterol (**4**) and Δ^7-stigmasterol (**5**); and the saponins 3-*O*-β-D-glucopyranosyl stigmasterol (**6**), 3-*O*-β-D-glucopyranosyl Δ^7-stigmasterol (**7**), 3-*O*-β-D-glucopyranosyl β-sitosterol (**8**), and 3-*O*-β-D-glucopyranosyl spinasterol (**9**). From the crude hexane extract the same steroids were identified.

All the isolated compounds were characterized by comparing their physical and spectroscopic properties, using 1D (^{1}H, ^{13}C, DEPT) and 2D NMR (TOCSY, HMQC and HMBC), MS (EI, ESI-MS or HREI-MS), IR and UV spectra, with those reported in the literature (Agrawal, 1989; Kojima *et al.*, 1990; Markham and Geiger, 1994; Harborne, 1996; Aquino *et al.*, 2001; Yayli *et al.*, 2001; Salvador *et al.*, 2006) or by HRGC and HPLC analysis complying with standard process.

Susceptibility test

Strains of microorganisms used

Susceptibility tests were performed using thirty strains of microorganisms (Table I) including Gram-positive (fifteen strains) and Gram-negative bacteria (four strains), yeasts (seven strains) and dermatophytes (four strains). These microorganisms were collected from Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão

Preto, Universidade de São Paulo (FCFRP/USP), Ribeirão Preto, SP, Brazil.

Determination of antimicrobial activity

Sensitivity tests were performed by an agar-well diffusion method (well technique in double layer) according to Stefanello *et al*. (2008), Salvador *et al*. (2004), Pujol *et al*. (1996), Espinel-Ingroff *et al*. (1995), and Grove and Randall (1955) with modifications. The test bacterial and fungal strains were inoculated into Mueller-Hinton medium (MH, Difco) agar plates (*Escherichia*, *Pseudomonas*, *Kocuria* and *Staphylococcus* strains), brain heart infusion (BHI, Difco) agar plates (*Enterococcus* and *Streptococcus* strains), and RPMI-1640 medium (Sigma) with MOPS (USB) buffer solutions in agar plates (*Candida* and *Trichophyton* strains), with an inoculum size of 10^6 cfu/mL (0.5 McFarland scale). The inoculum size of each test strain was standardized according to the National Committee for Clinical Laboratory Standards (NCCLS, 1993, 1998).

In the screening phase, aliquots of 20.0 μL of each test drug solution were applied in wells of 5.0 mm diameter. For these studies the solutions were prepared in propyleneglycol/RPMI-1640 medium (5:95) at 5.0 mg/mL for all crude extracts.

Table I. MIC values (μg/mL) of compounds **1**–**16** isolated from *Alternanthera tenella*.

Microorganism	Compound									
	1		**1–3**		**4–5**		**6–7**		**7**	
	H[a]	MIC[b]	H	MIC	H	MIC	H	MIC	H	MIC
Kocuria rhizophila (ATCC 9341)[c]	–	–	7	>500	8	>500	–	–	–	–
Staphylococcus aureus (ATCC 6538)[c]	–	–	7	>500	8	>500	7	>500	7	>500
S. aureus (ATCC 25923)[c]	–	–	–	–	–	–	–	–	–	–
S. aureus (ATCC 25213)[c]	–	–	–	–	–	–	–	–	–	–
S. aureus penicilinase + (7+)[d]	–	–	6	500	6	500	6	>500	6	>500
S. aureus penicilinase – (8–)[d]	–	–	–	–	–	–	–	–	–	–
Staphylococcus epidermidis (6ep)[d]	–	–	–	–	–	–	–	–	6	>500
Streptococcus mutans (ATCC 25175)[c]	7	500	7	100	7	500	–	–	–	–
S. mutans (Fab 3)[c]	6	500	6	100	7	500	–	–	–	–
S. mutans (11.1)[d]	9	>500	7	>500	7	>500	6	500	6	500
S. mutans (9.1)[d]	8	500	8	500	9	500	7	>500	7	>500
S. mutans (9.31)[d]	6	500	7	500	6	500	–	–	–	–
S. mutans (11.22.1)[d]	6	500	8	500	9	500	6	500	7	500
Streptococcus sobrinus (180.3)[d]	6	500	7	100	9	100	6	100	6	100
Enterococcus faecalis (ATCC 10541)[c]	–	–	–	–	–	–	–	–	–	–
Escherichia coli (ATCC 10538)[c]	6	>500	7	>500	6	>500	7	>500	7	>500
E. coli (ec 26.1)[d]	–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa (ATCC 27853)[d]	–	–	–	–	–	–	–	–	–	–
P. aeruginosa (290D)[d]	–	–	–	–	–	–	–	–	–	–
Candida albicans (ATCC 1023)[c]	–	–	7	>500	9	>500	–	–	–	–
C. albicans (ATCC 64548)[c]	–	–	–	–	–	–	–	–	–	–
C. albicans (cas)[d]	–	–	6	>500	7	>500	6	>500	6	>500
Candida tropicalis (ct)[d]	–	–	–	–	–	–	–	–	–	–
Candida glabrata (ATCC 90030)[c]	–	–	–	–	–	–	–	–	–	–
Candida krusei (ATCC 6258)[c]	–	–	–	–	–	–	7	500	7	500
Candida parapsilosis (ATCC 22019)[c]	–	–	–	–	–	–	–	–	–	–
Trichophyton rubrum (Tr 5)[d]	t	500	t	500	t	>500	–	–	–	–
T. rubrum (Tr 19)[d]	–	–	–	–	–	–	7	500	t	500
Trichophyton mentagrophytes (Tm 9)[d]	–	–	–	–	–	–	7	500	t	>500
T. mentagrophytes (Tm 17)[d]	–	–	–	–	–	–	–	–	–	–

[a] H, halo (diameter) of inhibition; [b] MIC, minimum inhibitory concentration; [c] standard strain; [d] field strain.
t, Trace of inhibition (trailing effect) after 6 days of incubation at 30 ºC.
–, Without inhibition of the development.

After holding the plates at room temperature for 2 h to allow diffusion of the test drug into the agar, they were incubated for 24 and 48 h at 37 ºC for the bacteria and yeast strains, respectively, and for 6 d at 30 ºC for the dermatophytes. For *Enterococcus* and *Streptococcus* strains the incubations were performed under microaerophilic conditions. The inhibition zone, corresponding to the halo (H) formed from the well edge to the beginning of the region of microbial growth, was measured in millimeters (mm). In these tests, gentamicin disks (10 μg), bacitracin (0.2 UI/mL) and ketoconazole (100 μg/mL) were used as experimental positive controls for the strains analyzed, while propyleneglycol/RPMI-1640 medium (5:95) served as the negative control.

The minimal inhibitory concentration (MIC) was determined for each isolated compound using broth microdilution techniques as described by the National Committee for Clinical Laboratory Standards (NCCLS, 1998, 2003) to give a concentration between 50 and 500 μg/mL. The MIC was calculated as the lowest concentration showing complete inhibition of a tested strain.

The tests were performed at least in duplicate for each microorganism evaluated, and the final results were presented as the arithmetic average.

Compound															
8–9		**10**		**11**		**12**		**13**		**14**		**15**		**16**	
H	MIC	H	MIC	H	MIC	H	MIC	H	MIC	H	MIC	H	MIC	H	MIC
–	–	–	–	–	–	7	500	–	–	–	–	–	–	–	–
6	>500	7	100	6	100	6	100	6	50	6	500	6	500	6	500
–	–	6	100	–	–	6	100	7	100	6	500	6	500	6	500
–	–	6	100	6	100	7	100	–	–	–	–	–	–	–	–
–	–	6	500	6	500	7	100	6	500	–	–	–	–	–	–
–	–	–	–	7	100	6	100	–	–	–	–	–	–	–	–
–	–	6	100	6	100	8	100	–	–	–	–	–	–	–	–
–	–	–	–	–	–	6	500	–	–	–	–	–	–	–	–
6	500	–	–	–	–	6	50	–	–	–	–	–	–	–	–
–	–	7	500	6	500	6	100	6	>500	–	–	–	–	–	–
7	100	6	500	6	100	6	>500	–	–	–	–	–	–	–	–
–	–	6	100	6	500	6	100	6	>500	–	–	–	–	–	–
7	500	7	500	6	500	6	500	6	>500	–	–	–	–	–	–
6	500	6	50	7	50	7	100	–	–	–	–	–	–	–	–
–	–	6	500	7	500	7	100	–	–	–	–	–	–	–	–
7	>500	9	>500	9	>500	7	>500	9	>500	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	>500	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	>500	8	500	8	500	6	500	7	100	–	–	–	–	–	–
7	500	7	500	7	500	6	500	–	–	–	–	–	–	–	–
–	–	–	–	–	–	6	>500	7	100	6	>500	6	>500	6	>500
10	100	8	500	20	500	7	500	7	500	–	–	–	–	–	–
7	500	8	500	8	500	–	–	–	–	–	–	–	–	–	–
7	>500	7	500	7	500	7	500	7	100	–	–	–	–	–	–
–	–	–	–	8	500	–	–	6	500	–	–	–	–	–	–

Results and Discussion

Here we report, for the first time, the antibacterial and antifungal potential of the callus culture and adult plant extracts of *A. tenella*, as well as the isolation of the steroids, saponins and flavonoids displaying *in vitro* antimicrobial activities.

The organic crude extracts from a callus culture and adult plants of *A. tenella* were considerably active against the microorganisms evaluated, while the aqueous lyophilized extracts from adult plants prepared in hot and cold water did not interfere appreciably (at 5.0 mg/mL) with the growth of the microorganisms tested.

The hexane and ethanol extracts obtained from the *A. tenella* adult plants collected during the same period of the years 1997 and 2002 (collections 1 and 2) and the extracts from a callus culture obtained from two different hormonal combination media (T11 and T43 medium) showed antimicrobial activity. It was found that the adult plant extracts from two different collections (1 and 2) were bioactive against Gram-positive and Gram-negative bacteria, yeasts, and dermatophytes, with an antimicrobial activity mainly associated with Gram-positive bacteria, yeasts, and filamentous fungi. The adult plants extracted with ethanol and hexane were active against twenty strains of microorganisms with predominance for *Staphylococcus*, *Streptococcus*, and *Trichophyton*. The inhibition halo produced by organic extracts from adult plants were in the range 6–16 mm.

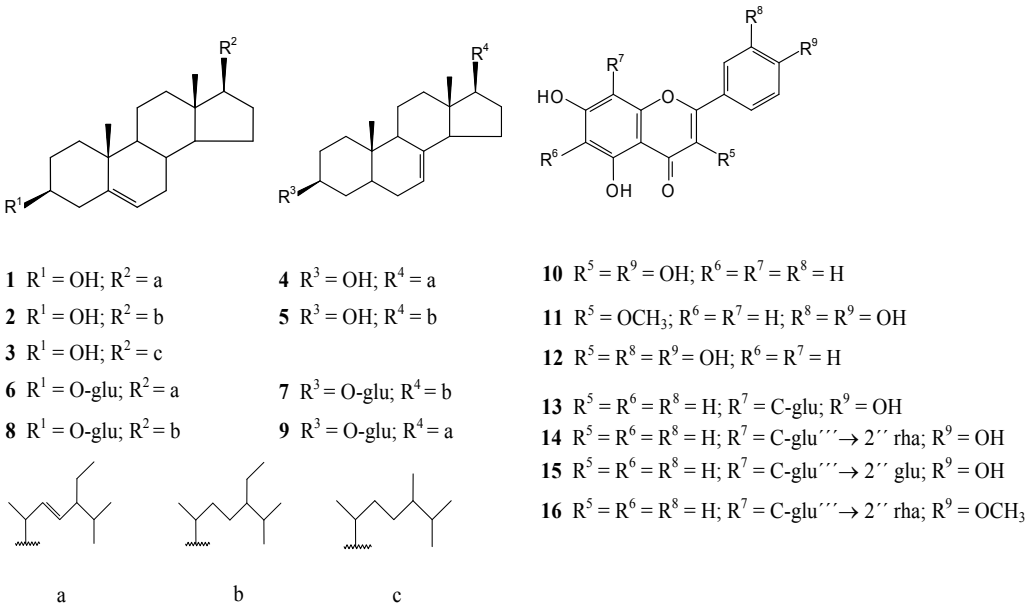
The plant cell callus culture extracts (AtT11 and AtT43) with two different hormonal combinations were bioactive as well with a bioactivity, in general, against the same strains as the adult plant extracts, with a low value of inhibition halo, in the range 6–10 mm. This fact may have happened due to the presence of the antimicrobial compounds in the plant cell callus culture in lower concentration; this hypothesis needs to be evaluated. On the other hand, in two cultures (AtT11 and AtT43) between 60–80% of the *A. tenella* leaf explants formed calli and showed similar antibacterial and antifungal activity despite the different hormonal composition of the culture medium. Therefore, the maintenance of the antimicrobial activity for crude extracts of *A. tenella* adult plants and of plant cell callus culture was verified. These results are in accordance with results reported for other plants with antimicrobial activity, for example by Thien and Goslinska (2002) for a culture of *Solidago virgaurea* L. and by Salvador *et al.* (2003) for a culture of *A. maritima*. On the other hand, the extracts assayed were ineffective against *E. faecalis* (ATCC 10541), *E. coli* (strains ATCC 10538 and ec 26.1), *P. aeruginosa* (strains ATCC 27853 and 290D), *C. albicans* (strains ATCC 1023, 64548 and cas), and *C. parapsilosis* (ATCC 22019).

Gentamicin (10 μg/disk) and bacitracin (0.2 UI/mL), used as a positive experimental control against all bacterial strains assayed, produced halos of inhibition of 22–32 mm, while ketoconazole (100 μg/mL) served as the positive experimental control for all fungal strains assayed with halos of inhibition of 12–35 mm. The medium containing propyleneglycol/RPMI-1640 medium (5:95) was used as negative control for which no inhibitory effect could be observed.

In a phytochemistry study using bioassay-guided fractionation, steroids, saponins and flavonoid aglycones and C-glycosides were isolated. MIC values for the isolated constituents varied from 50 to 500 μg/mL (Table I). The chemical structures of the steroids, flavonoids and saponins isolated from *A. tenella* showing biological activity are presented in Fig. 1.

The structures of the steroids stigmasterol (**1**), β-sitosterol (**2**), campesterol (**3**), spinasterol (**4**) and Δ^7-stigmasterol (**5**) were confirmed by comparison of their NMR spectral data with those reported in the literature (Kojima *et al.*, 1990; Chaurasia and Swichtl, 1987) and by GC analysis using standard samples.

The bioactive saponins **6**, **7**, **8** and **9** were identified by comparison of the spectral data with published ones. The observation of several signals attributed to methyl, methylene and olefinic groups in the ^{1}H and ^{13}C NMR spectra suggested the presence of a saponin and a mixture of saponins. The stigmasteryl and stigmasteryl-7-en-3-β-ol moieties as well as spinasteryl and β-sitosteryl moieties were identified by the shifts of the olefinic carbon atoms (Kojima *et al.*, 1990). The peaks at δ_H 4.87 (J = 7.7 Hz, confirming the β-position of the sugar) and δ_C 102.1 and δ_C 102.4 in the ^{1}H and ^{13}C NMR spectra indicated glucose for a mixture of 3-*O*-β-D-glucopyranosyl stigmasterol (**6**) and 3-*O*-β-D-glucopyranosyl Δ^7-stigmasterol (**7**) glucopyranosides. For the mixture 3-*O*-β-D-glucopyranosyl β-sitosterol (**8**) and 3-*O*-β-D-glucopyranosyl spinasterol (**9**) glucopyranosides, the peaks at δ_H 4.97 (J = 3.6 Hz, confirming the



1 $R^1 = OH$; $R^2 = a$
2 $R^1 = OH$; $R^2 = b$
3 $R^1 = OH$; $R^2 = c$
4 $R^3 = OH$; $R^4 = a$
5 $R^3 = OH$; $R^4 = b$
6 $R^1 = O\text{-glu}$; $R^2 = a$
7 $R^3 = O\text{-glu}$; $R^4 = b$
8 $R^1 = O\text{-glu}$; $R^2 = b$
9 $R^3 = O\text{-glu}$; $R^4 = a$

10 $R^5 = R^9 = OH$; $R^6 = R^7 = R^8 = H$
11 $R^5 = OCH_3$; $R^6 = R^7 = H$; $R^8 = R^9 = OH$
12 $R^5 = R^8 = R^9 = OH$; $R^6 = R^7 = H$
13 $R^5 = R^6 = R^8 = H$; $R^7 = C\text{-glu}$; $R^9 = OH$
14 $R^5 = R^6 = R^8 = H$; $R^7 = C\text{-glu}''' \rightarrow 2'' \text{ rha}$; $R^9 = OH$
15 $R^5 = R^6 = R^8 = H$; $R^7 = C\text{-glu}''' \rightarrow 2'' \text{ glu}$; $R^9 = OH$
16 $R^5 = R^6 = R^8 = H$; $R^7 = C\text{-glu}''' \rightarrow 2'' \text{ rha}$; $R^9 = OCH_3$

Fig. 1. Chemical structures of the compounds with antimicrobial activity isolated from *Alternanthera tenella*.

α-position of the sugar) and δ_C 102.4 and δ_C 102.0 for the carbinolic carbon atom of glucose were observed.

The identities of the bioactive flavonoids **10**–**16** were confirmed by comparing their physical and spectroscopic properties, using 1D (^{1}H, ^{13}C, DEPT) and 2D NMR (TOCSY, HMQC and HMBC), ES-MS, IR and UV spectra, with those reported in the literature (Agrawal, 1989; Zhou *et al*., 1988; Harborne, 1996; Markham and Geiger, 1994; Aquino *et al*., 2001; Yayli *et al*., 2001; Salvador *et al*., 2006).

We reported the isolation of antibacterial and antifungal compounds from *A. tenella* by using bioassay-guided fractionation. Steroids, saponins and flavonoids (aglycones and C-glycosides) with antimicrobial activity were isolated from *A. tenella* extracts (Table I, Fig. 1). The results documented here indicate that the compounds isolated from *A. tenella* display antimicrobial activity and are in accordance with other previous studies reporting antimicrobial activities for flavonoids, saponins and steroids (Otsuka *et al*., 2008; Cushnie and Lamb, 2005; Salvador *et al*., 2004; Panizzi *et al*., 2002; Rauha *et al*., 2000; Chattopadhay *et al*., 2001; Tereschuk *et al*., 1997). Thus, our results explain and justify, at least in part, the popular use of *A. tenella* for the treatment of infections. However, further investigations are necessary to confirm the antimicrobial potential of this plant and constituents looking toward a pharmaceutical employment.

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