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Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. ex Hook et Arn

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Abstract

The purpose of the present study was to investigate the antibacterial activity of seven ethanolic extracts and three aqueous extracts from various parts (leaves, stems and flowers) of *A. aroma* against 163 strains of antibiotic multi-resistant bacteria.

The disc diffusion assay was performed to evaluate antibacterial activity of the *A. aroma* crude extracts, against several Gram-positive bacteria (*E. faecalis, S. aureus*, coagulase-negative stahylococci, *S. pyogenes, S. agalactiae, S. aureus* ATCC 29213, *E. faecalis* ATCC 29212) and Gram-negative bacteria (*E. coli., K. pneumoniae, P. mirabilis, E. cloacae, S. marcescens, M morganii, A. baumannii, P. aeruginosa, S. maltophilia, E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922). All ethanolic extracts showed activity against gram-positive bacteria. Among all obtained extracts, only leaf and flower fluid extracts showed activity against Gram-negative bacteria. Based on this bioassay, leaf fluid extracts tended to be the most potent, followed by flower fluid extracts. Minimal inhibitory concentration (MIC) values of extracts and antibiotics were comparatively determined by agar and broth dilution methods. Both extracts were active against *S. aureus*, coagulase-negative stahylococci, *E. faecalis* and *E. faecium* and all tested Gram-negative bacteria with MIC values from 0.067 to 0.308 mg/ml. In this study the minimal bactericidal concentration (MBC) values were identical or twice as high than the corresponding MIC for leaf extracts and four or eight times higher than MIC values for flower extracts. This may indicate a bactericidal effect. Stored extracts have similar antibacterial activity as recently obtained extracts.

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The *A. aroma* extracts of leaves and flowers may be useful as antibacterial agents against Gram- negative and Gram-positive antibiotic multi-resistant microorganisms.
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Keywords: *Acacia aroma*; Antimicrobial activity; Tusca; Gram-negative bacteria; Gram-positive bacteria; Antibiotic-resistant

Introduction

The genus *Acacia*, one of the important genera of the family *Fabaceae* (Sub family *Mimosaceae*) includes aproximately 1350 species and is abundant in Australia, Africa, India and America. The *Acacia* species are of immense value for reforestation and reclamation of wastelands (Skolmen, 1986), for fuel wood, timber, shelter belts and soil improvement (Palmberg, 1981). Most of its species yield excellent firewood and some are rich sources of protein, tannin, paint, ink, flavouring agents, pulpwood and gum. From the ecological point of view, they can adapt to extremes of temperature, moisture stress and therefore, can be grown in both arid and moist regions in a wide range of tropical soils. Some are also engaged in symbiotic relationship with *Rhizobium* and mycorrhizal fungi thereby increasing soil fertility. A number of secondary metabolites have been reported from various *Acacia* species including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non protein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003). However, the secondary metabolites of only a small proportion of *Acacia* species have been examined in detail. Cytotoxic activities have been reported for *Acacia angustissima* and *Acacia pennatula* (Popoca et al., 1998). Otherwise, *Acacia nilotica* has been traditionally used for ailments such as diarrhea and was reported to have antihyperglycemic (Akhtar and Khan, 1985), antimicrobial (Abd-El-Nabi, 1992), molluscicidal (Hussein, 1984), antihypertensive and antiplatelet aggregatory activities (Shah et al., 1997). A potent cyclooxygenase-1 inhibition by extracts of *A. ancistrocarpa* was observed (Li et al., 2003).

Acacia aroma Gill.ex Hook et Arn, common name tusca (Burkart, 1952), a native species of Argentina, is member of genus *Acacia* subgenus *Acacia*, widely distributed in the provinces of Tucumán, Salta, Santiago del Estero, Catamarca, La Rioja, Formosa, Chaco, Córdoba, San Luis and Santa Fe. *A.aroma* belongs to the phyto-geographical formation of the Neotropical region, Provincia Chaqueña (Cabrera, 1971). This plant is used in Argentine folkloric medicine as wound healing, antiseptic and for the treatment of gastrointestinal disorders. Leaf and bark infusions have diuretic, anti-inflammatory and cicatrizant uses. Its fruits are important components in the diet of the animals living in this area. Available literature indicates that no previous studies have been carried out on the biological properties of this plant. Phytochemistry studies on *Acacia aroma* indicated that the main components of the steam volatile flower oils were methyl salicylate and eugenol (Lamarque et al., 1998). Furthermore, fatty acid profiles of *A. aroma* seed were examined (Lamarque et al., 2000).

Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Scrinivasan et al., 2001; Kumarasamy et al., 2002; Ali et al., 2001; Masika and Afolayan, 2002; Hamill et al., 2003). At present, staphylococci and enterococci

among Gram-positive bacteria, some enterobacteria and non fermenter organisms such as *Pseudomonas aeruginosa* and *Acinetobacter* spp, are recognized as important nosocomial pathogens.

The purpose of the present study was to investigate the antimicrobial activity of leaf, stem and flower extracts of *A. aroma* against Gram-positive and Gram-negative bacteria. The selected bacteria were antibiotic resistant or multi-resistant human pathogens. The extracts with the highest antibacterial effectiveness were chosen for subsequent use in pharmaceutical formulations.

Materials and methods

Plant material

The plants used for the present study were collected from September to March in Trancas, Tucumán, Argentina. For future reference, voucher specimens were deposited in the Herbarium of the Institute de Estudios Vegetales (IEV), Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina). The parts used were leaves, stems and flowers.

Preparation of Acacia aroma extracts

Lixiviation (fluid extract): ground air-dried plant material was extracted with a 60% or 70% ethanol (v/v) solvent for leaves and flowers, using a percolator apparatus at room temperature until total extraction (Farmacopea Argentina 6th ed., 1978).
Maceration (tincture): ground air-dried plant material was macerated in ethanol (1 g of dry tissue per 5 ml of 60% ethanol for leaves and stems or 70% ethanol for flowers) for 7 days under shaking (40 cycles/min) at room temperature.
Alcoholature: fresh plant material was cut in small pieces and macerated in ethanol (1 g of tissue per 1 ml of 60% or 70% ethanol for leaves or flowers, respectively) for 8 days under shaking (40 cycles/min) at room temperature.

In all cases the extracts were filtered through Whatman No. 4 filter paper, dried under reduced pressure at 40 °C, and weighed.

Decoction: dried and powdered plant material (5 g) was boiled with 70 ml of water for 20 min. After cooling to 40–45 °C, the liquid was filtered and the volume adjusted to 100 ml with distilled water.

The aqueous extracts were lyophilized.

Microorganisms and media

Clinical isolates of the following organisms: *Staphylococcus aureus* (n = 46), *Stahylococcus* spp (n = 6), *Enterococcus faecalis* (n = 57), *Enterococcus faecium* (n = 1), *Streptococcus agalactiae* (n = 1), *Streptococcus pyogenes* (n = 1), *Escherichia coli* (n = 14), *Klebsiella pneumoniae* (n = 7), *Proteus mirabilis* (n = 6), *Enterobacter cloacae* (n = 9), *Serratia marcescens* (n = 2), *Morganella morganii* (n = 2), *Acinetobacter baumannii* (n = 2), *Pseudomonas aeruginosa* (n = 7), *Stenotrophomonas maltophilia*

(n = 2) were recovered between January 1999 and December 2000 from clinical samples obtained from the Hospital Nicolás Avellaneda, San Miguel de Tucumán, Tucumán, Argentina. The following reference strains were included in the study: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922.

The strains were identificated by the use of Biochemical profiles according to the recommendations of the Manual of Clinical Microbiology (Murray et al., 1999). All organisms were maintained in brain-heart infusion (BHI medium) containing 30% (v/v) glycerol at − 20 °C. Before testing, the suspensions were transferred to trypticase soy agar supplemented with 5% of sheep blood (Difco) and aerobically grown overnight at 35 °C. Individual colonies were isolated and suspended in 5 ml of 0.9% NaCl solution. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard and diluted in CAMHB (cation - adjusted Müeller–Hinton broth) in order to achieve the adequate inoculum in each case.

The cell number in CAMHB was estimated using a serial dilution technique (NCCLS, 2002) for each assay.

Disk diffusion method

Petri dishes (9 cm in diameter) were prepared with 10 ml of a base layer of Müeller–Hinton agar medium (MHA, Laboratorio Britania, Argentina) and a top layer (3 ml) of 0.2% BHI agar medium inoculated with 30 μl of each bacterial suspension (10^5 bacteria × ml^{-1}). After drying in a sterile hood, 6 mm diameter disks soaked with 15 μl of the different extract dilutions were placed on the agar. Disks containing Imipenem, meropenem, cefotaxime, ceftacidime, vancomycin, oxacillin, ampicillin, gentamicin and alcohol were used as controls. The dishes were incubated at 35 °C for 16–20 hours.

All tests were performed in duplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination

1) *Serial agar macrodilution method*: The tests were performed in MHA medium. Serial two-fold dilution of each extract were added to an equal volume of the medium. Control dishes containing the same volume of ethanol or distilled water were made. After cooling and drying, the plates were inoculated in spots of 2 μl with each bacterial cell suspension (1×10^4 cfu) and incubated aerobically for 16–20 hours at 35 °C. A growth control of each tested strain was included.

MIC was defined as the lowest concentration of *A.aroma* extract at which no colony was observed after incubation.

2) *Broth microdilution method* was used to determine the MIC and the MBC of the *A. aroma* extracts against the test organisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). This test was performed in sterile 96-well microplates. The ethanolic extracts were properly prepared and transferred to each microplate well in order to obtain a twofold serial dilution of the original extract (from 1:2 to 1:128 starting from the concentration of 1000 μg/ml). The inocula (100 μl) containing 5×10^5 cfu of each microorganism were added to each well. A number

of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added), and the ethanol inhibitory effect. Plates were aerobically incubated at 35°C. After incubation for 16–20 hrs, bacterial growth was assayed by absorbance measurement at 625 nm. Bacterial growth was also indicated by the presence of turbidity and a pellet on the well botton.

MIC was defined as the lowest concentration of *A. aroma* extract that had restricted growth to a level <0.05 at 625 nm (no macroscopically visible growth).

To confirm MICs and to establish MBC, 10 μl of each culture medium with no visible growth was removed from each well and inoculated in blood agar or MHA plates. After 16–20 hrs of aerobic incubation at 35°C, the number of surviving organisms was determined.

MBC was defined as the lowest extract concentration at which 99.9% of the bacteria were killed. Each experiment was repeated at least three times.

The MIC values were also determined for levofloxacine, piperacillin/tazobactam, imipenem, meropenem, ceftriaxone, cefotaxime, ceftacidime, cefuroxime, cefepime, amikacin and ampicillin/sulbactam for Gram-negative bacteria and oxacillin, streptomycin, ampicillin, methicillin, gentamicin and vancomycin for Gram-positive bacteria. The antimicrobial agents were supplied by Sigma Chemical Co (USA) and Laboratorio Britania S.A. Argentina.

Phytochemical screening

The components of the different extracts (200 μg) were separated by TLC (Kieselgel 60 F254 0.2 mm, Merck). Toluene: ethyl acetate: formic acid (5:4:0.5); ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26); chloroform:methanol (9:1) and chloroform:methanol:water (8:2:1) were used as development solvents. The separated components were visualized under ultraviolet light (254 and 360 nm, UV Lamp Model UV 5L-58 Mineralight Lamp) and sprayed with 1% ferric trichloride, natural product reagent (1% methanolic 2-aminoethyl diphenylborate) (Wagner et al., 1984) or aluminium chloride for phenolic compounds, methanolic potassium hydroxide for coumarins (Harbone, 1973), Dragendorff's reagent for alkaloids and anisaldehyde/sulfuric acid for steroids and terpenes (Krebs et al., 1969).

Bioautography

Developed (chloroform: methanol, 9:1, as solvent)TLC plates were dried overnight in a sterile room. Then, plates were covered with 2 ml of soft medium (BHI with 0.6% agar) containing 10^5 cfu of *S. aureus* (F7), *E. faecalis* (F213) or *P. aeruginosa* (F305), incubated at 35°C for 16–20 hs and sprayed with a 2.5 mg/ml MTT solution (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) in PBS (10 mM sodium phosphate buffer, pH 7, with 0.15 M NaCl). Plates were incubated at 35°C for 1 h in the dark for colour development. The growth inhibition areas, yellow coloured, were compared with the Rf of the related spots on the TLC plate revealed with different reagents.

Phenolic compound determinations

Total phenolic compound content was determined using the Folin–Ciocalteau method (Singleton et al., 1999). Results were expressed as coumarin equivalents.

Results

Crude extracts of *Acacia aroma* prepared according to Farmacopea Argentina (decoction, tincture, fluid extract and alcoholature) were compared for their antibacterial activity.

All extracts were analyzed for total phenolic compound content. The yield of water and ethanol soluble principle extractions was determined (Table 1).

Antibacterial activity by agar diffusion assay

The antibacterial activity of *A. aroma* extracts was assayed in vitro by agar diffusion method against ten clinical isolates chosen by their largest antimicrobial resistance profiles.

Table 2 summarizes the microbial growth inhibition by seven crude ethanolic extracts and three crude aqueous extracts of *A. aroma*. Crude ethanolic extracts of leaves, stem and flowers were particularly active against Gram-positive bacteria (*E. faecalis, S. aureus, S. pyogenes*). Only tincture and alcoholature of flowers and decoction of stems showed good activity with *S. agalactiae*. The bacterium growth inhibition produced by *A. aroma* extracts varied in relation to the type of extract and to the bacterium strain used. The most active against Gram-negative bacteria were tincture and fluid extracts of leaves and flowers.

Susceptibility of Gram-positive bacterium species to A. aroma extracts

In order to determine the comparative efficacy of antibiotics and *A. aroma* extracts against Gram positive bacteria, each extract was tested against 52 and 58 recently clinical isolates of *Staphylococcus* spp and *Enterococcus* spp respectively. These isolates included 23% MRSA (methicillin-resistant *S. aureus*), 65.38% MSSA (methicillin-sensitive *S. aureus*), 1.92% MRCNS (methicillin resistant coagulase-negative staphylococci) and 9.61% MSCNS (methicillin sensitive coagulase-negative staphylococci). Most of the MRSA clinical isolates were highly resistant not only to methicillin but also to many other antimicrobial agents, whereas the MSSA strains were susceptible to nearly all of them. All MRSA strains were uniformly inhibited by 1 μg/ml or less vancomycin (Craven, 1989). All *Enterococcus* spp

Table 1
Ethanol and water soluble principles and total phenolic compound content in *A. aroma* extracts

Extracts	Plant material	Soluble Principles[a] (mg/ml)	Phenolic compounds[b] (mg/ml)
Tincture	Leaves	29.95	1.70
	Stem	21.90	3.14
	Flowers	23.45	1.05
Fluid extract	Leaves	178	10
	Flowers	132	10
Alcoholature	Leaves	48.45	2.60
	Flowers	25.20	0.70
Decoction	Leaves	8.5	0.55
	Flowers	8.9	0.60
	Stem	10	0.30

[a] Water and ethanolic soluble principles/ ml of extract.
[b] Phenolic compound content/ ml of extract.

Table 2
Activity of different extracts of *A.aroma* against some microbial agents

Acacia aroma Preparation	Plant parts	Microorganisms									
		1	2	3	4	5	6	7	8	9	10
Tincture	Leaves	+++	++	0	++	+	0	0	0	0	0
	Flowers	+++	++	++	++	++	0	0	+	0	0
	Stems	++	++	0	++	0	0	0	0	0	0
Fluid extract	Leaves	+++	+++	0	+++	+++	+++	0	0	0	0
	Flowers	+++	+++	0	+++	+++	+++	0	0	0	+++
Alcoholature	Leaves	+	+++	++	++	0	0	0	+++	++	0
	Flowers	++	+++	++	++	0	0	0	0	++	0
Decoction	Leaves	+	+	0	+	0	+	0	0	0	0
	Flowers	+	+	0	+	0	+	0	0	0	0
	Stems	++	0	++	+	0	+	0	0	0	0

The inhibition is reported as 0, without inhibition; +, dr < 1.00; ++ dr > 1.00 < 1.50; +++ dr > 1.50, where dr is the diameter of the inhibition zone (cm) at the same phenolic compound concentrations for all *A. aroma* preparation.
1 *S. aureus*; 2 *S. pyogenes*; 3 *S. agalactiae; 4 E. faecalis*; 5 *P. aeruginosa*; 6 *S. marscences*; 7 *E. coli*; 8 *A. baumanni*; 9 *K. pneumoniae*; 10 *S. maltophilia*.

isolates were susceptible to vancomycin and 90% of them were also susceptible to ampicillin, 64,6% and 30% were resistant to a high level of gentamicin and streptomycin, respectively. None of the isolates were β-lactamase producers.

Table 3 shows the in vitro growth inhibitory activity of leaf and flower fluid extracts against clinical isolates of *Staphylococcus* spp. and *Enterococcus* spp. Both extracts inhibited the growth of *Staphylococcus* spp. and *Enterococcus* spp. at concentrations between 67 to 308 μg/ml.

The leaf extract concentrations required to obtain a bactericidal effect against *Staphylococcus* spp. and *Enterococcus* spp were the same or two fold higher than the corresponding MIC values. The

Table 3
Antimicrobial activity of leaf and flower fluid extracts against Gram-positive bacteria

	Leaf fluid extract		Flower fluid extract	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Staphylococcus species				
MRSA (n = 12)	125 ± 0.00	250 ± 55.00	250 ± 58.13	250 ± 58.13
MSSA (n = 34)	125 ± 58.00	125 ± 58.00	250 ± 55.50	250 ± 58.13
MSCNS (n = 5)	250 ± 58.50	250 ± 58.50	250 ± 55.60	568 ± 123
MRCNS (n = 1)	250 ± 0.00	250 ± 0.00	250 ± 45.60	568 ± 123
ATCC 29213	125 ± 0.00	125 ± 0.00	125 ± 0.00	250 ± 0.00
Enterococcus species				
AR (n = 6)	250 ± 58	250 ± 58	500 ± 120	1500 ± 500
AS (n = 52)	250 ± 0.00	250 ± 58	250 ± 38	1500 ± 500
ATCC 29212	250 ± 0.00	750 ± 250	250 ± 38	1500 ± 500

n: Strain number.
AR: Ampicillin resistant; AS: Ampicillin susceptible.
Results are the mean of MIC or MBC values followed by the standard deviation.

MBC values for flower extracts were two or four times higher than the corresponding MIC for both assayed bacteria. Stored (1 year at 7°C) and recently prepared extracts had similar antibacterial activity.

Susceptibility of Gram-negative bacteria to A. aroma extracts

The effect of *A. aroma* extracts on isolates of extended-spectrum cephalosporin-resistant Gram-negative bacteria were evaluated. The isolated strains included *K.pneumoniae* cefotaxime-resistant (MICs greater than or equal to 124 mg/l); *E. cloacae, E. coli; S. marcescens* cefotaxime-resistant (MICs greater than or equal to 64 mg/l); *P. aeruginosa* and *A. baumannii* ceftazidime-resistant (MICs greater than or equal to 32 mg/l). Imipenem and meropenem were effective against the cefotaxime-resistant Enterobacteriaceae tested (>90% of isolates were susceptibles). However meropenem (carbapenems) showed limited activity against the ceftazidime -resistant strains.

Leaf and flower fluid extracts of *A. aroma* showed good activity against Gram-negative bacteria (*S. marcescens* and *P. aeruginosa*) by agar diffusion. However, because of its reliance on the solubility and the rate of extract diffusion, low zones of inhibition could often falsely arise. Further tests using agar and broth dilution assays were made to determine the MIC and MBC of leaf and flower fluid extracts against Gram-negative bacteria (Table 4). All assayed bacteria were sensitive to them with MIC values of 125 to 305 μg/ml. Leaf extract concentrations required to obtain a bactericidal effect against all assayed isolates were the same or two fold higher than MIC values. Otherwise, for the flower fluid extract, the MBC values were four to eight fold higher than MIC values. The more sensitive were ceftazidime-resistant isolates, *P. mirabilis, M. morgani* and *S. maltophilia*.

Table 4
MIC and MBC (μg/ml) of leaf fluid extracts against Gram-negative pathogenic bacteria isolated from skin, blood or respiratory tract infections and reference strains

Strains	n	Leaf fluid extracts		Flower fluid extracts	
		MIC	MBC	MIC	MBC
Escherichia coli	14	246 ± 46	425 ± 247	214 ± 30	750 ± 280
Klebsiella pneumoniae	7	250 ± 0.0	500 ± 0.0	235 ± 24	1500 ± 500
Proteus mirabilis	6	175 ± 82	175 ± 82	216 ± 26	241 ± 37
Enterobacter cloacae	9	233 ± 50	383 ± 125	233 ± 35	572 ± 320
Serratia marcescens	2	250 ± 0.0	250 ± 0.0	250 ± 0.0	1000 ± 0.0
Morganella morganii	2	250 ± 0.0	125 ± 0.0	250 ± 35	500 ± 35
Acinetobacter baumannii	2	125 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0
Pseudomonas aeruginosa	7	250 ± 53	125 ± 18	250 ± 39	289 ± 47
Stenotrophomonas maltophilia	2	125 ± 0.0	125 ± 0.0	250 ± 0.0	250 ± 0.0
Escherichia coli ATCC 35218	1	250 ± 0.0	250 ± 0.0	250 ± 0.0	1000 ± 0.0
Escherichia coli ATCC 25922	1	250 ± 0.0	250 ± 0.0	250 ± 0.0	1000 ± 0.0
Pseudomonas aeruginosa ATCC 27853	1	250 ± 55	250 ± 55	250 ± 55	1500 ± 500

n: Strain number.
Results are the mean of MIC or MBC values followed by the standard deviation.

Phytochemical screening

To have an idea of possible classes of compounds present in the more active extracts, a phytochemical analysis was carried out by observing coloured spots revealed after TLC. Different patterns of chemical components were observed. TLC analysis showed the presence of flavonoids in all the extracts which developed an orange and red colors by spraying a natural product reagent, indicative of 3′,4′ dyhydroxyflavonols and 3′,4′, 5′- trihydroxyflavonols (Bilia et al., 1996), while terpenes were detected in flower extracts (violet spots). No alkaloids were detected in either extract.

Contact bioautography, used for qualitative antibacterial activity detection indicated that the more active crude extracts possess many antibacterial compounds. With this assay, leaf alcoholic extracts showed four major antibacterial components (Rf of 0.22 ; 0.44; 0.62 and 0.88) against *S. aureus*. The leaf fluid extract showed three components (Rf of 0.22; 0.58 and 0.78) active against *E. faecalis*. These extracts contained phenolic compounds with Rf 0 that showed strong activity against *P. aeruginosa*. The majority of the spots correspond to flavonoid components. Flower fluid extracts and tincture showed three and one components with antibacterial activity against *S. aureus*, respectively

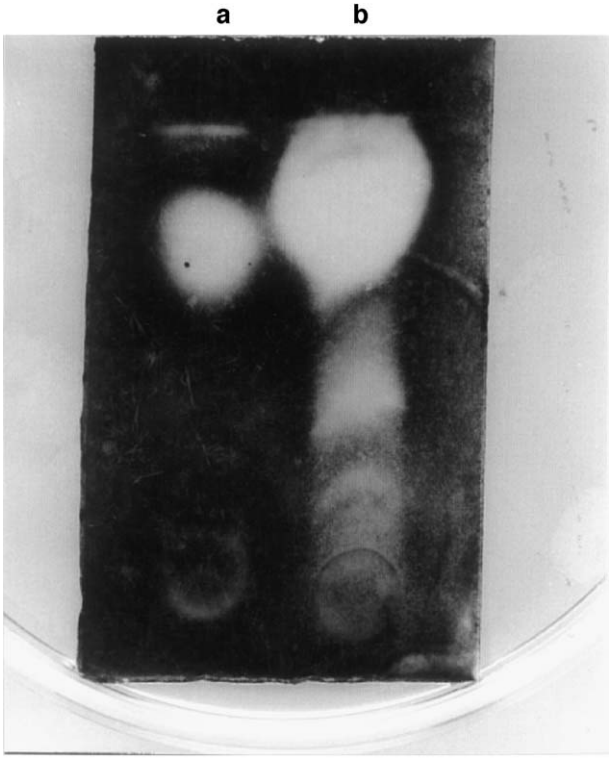

Fig. 1. Comparative bioautography of *A.aroma* flower extracts. Lane a: tincture; Lane b: fluid extract (200 μg of phenolic compounds of each extract/plate) was separated by TLC (Merck Kieselgel 60 F254) using chloroform/methanol (9:1) as eluant. 2 ml of soft medium (0.6% BHI agar) containing 10^5 CFU (colony forming units)/ml of *S. aureus* (F7) were added and incubated at 35°C for 24 h. Then, the plates were revealed.

(Fig. 1) and four antibacterial components for *E. faecalis* (Rf of 0.4; 0.6; 0.65 and 0.95) but the TLC separated chemical components were not active against *P. aeruginosa*, only the phytocomplex showed antibacterial activity.

Discussion

The obtained results indicate a difference in antimicrobial activity among the extracts. The agar diffusion bioassay showed that leaf and flower fluid extracts have the highest activity against all Gram-positive bacteria and they also showed good activity against Gram-negative bacteria. This is in agreement with observations by other authors (Ali et al., 2001; Masika and Afolayan, 2002; Chattopadhyay et al., 2002). The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). Based on MIC values, leaf fluid extract followed by flower fluid extract showed to be the most potent inhibitors against different Gram-positive species, including those resistant to many antimicrobial agents. MICs of the tested extracts show bacteriostatic activity between 67 to 308 μg/ml against Gram-positive bacteria. The MICs for Gram-negative strains were 125 to 305 μg/ml. Although the MICs obtained with the ethanolic extracts are high compared with those of imipenem (0.25 to 16 μg/ml), these results are of interest since they have been obtained with crude extracts and are not a pure product and it could be considered to have a good potency level (Rios et al., 1988).

Phytochemical studies of leaves and barks of other *Acacia* species revelead the presence of flavonol and flavone glycosides, aglycones, flavan 3 ols, flavan-3,4-diols and condensed tannins (Seigler, 2003). According with our results, *Acacia aroma* leaf fluid extracts showed a high flavonoid content. From the bioautography results leaf fluid extracts showed well-defined growth inhibition bands in correspondence with those of the flavonoids, both for Gram-negative and Gram-positive bacteria. Flower fluid extracts showed inhibition bands against Gram positive bacteria in correspondence with flavonoids and terpenes. Otherwise, the phytocomplex are necessary to obtain inhibition of Gram negative bacteria growth, suggesting the synergic action of several compounds.

The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to the evaluation of possible synergism among extract components for their antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

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