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Screening of anti-bacterial activity of medicinal plants from Belize (Central America)

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Abstract

Twenty-one extracts from seven herbal drugs, *Aristolochia trilobata* (Aristolochiaceae) leaves and bark, *Bursera simaruba* (Burseraceae) bark, *Guazuma ulmifolia* (Sterculiaceae) bark, *Hamelia patens* (Rubiaceae) leaves and *Syngonium podophyllum* (Araceae) leaves and bark, used in traditional medicine of Belize (Central America) as deep and superficial wound healers, were evaluated for their anti-bacterial properties. Activity was tested against standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. Almost all the extracts were able to inhibit the growth of one or more of the bacterial strains, except that of *Enterococcus faecalis*. For the first time an anti-microbial activity is reported for *Aristolochia trilobata* as well as for *Syngonium podophyllum*. The hexane extracts of *Aristolochia trilobata* leaves and bark were the most active extracts against *Staphylococcus aureus* (MIC = 0.31 and 0.625 mg/ml, respectively).

Keywords: Anti-bacterial activity; *Aristolochia trilobata*; *Bursera simaruba*; *Guazuma ulmifolia*; *Hamelia patens*; *Syngonium podophyllum*

1. Introduction

Due to the poor sanitary conditions and to the climate, often characterized by very hot temperature and high humidity, the infections of deep and superficial wounds are common in tropical developing countries. Therapy with synthetic antibiotics is not always possible due to their high cost. To overcome this problem, people use preparations obtained from plants growing in their countries following folk tradition, but without any scientific support.

Seven herbs, already studied as topical anti-inflammatory agents (Sosa et al., 2002), used in traditional medicine of Central America for deep and superficial wounds healing (Balick and Arvigo, 1998) were studied to validate their anti-microbial properties. Hexane, chloroform and methanol extracts of *Bursera simaruba* (Burseraceae) bark, *Aristolochia trilobata* (Aristolochiaceae) leaves and bark, *Guazuma ulmifolia* (Sterculiaceae) bark, *Hamelia patens* (Rubiaceae) leaves and *Syngonium podophyllum* (Araceae) leaves and bark were tested against standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, species often involved in skin infections.

2. Materials and methods

2.1. Plant material and extracts preparation

Plants were collected in February 1999 in Belize (Central America) and authenticated by Prof. M.J. Balick. All voucher specimens were dried and deposited at the New York Botanical Garden.

Air-dried and powdered plant materials, approximately from 200 to 500 g were submitted to sequential maceration with 2500 ml of *n*-hexane, chloroform and methanol at room temperature. After each step, the extracts were filtered and

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Table 1
In vitro anti-bacterial activity of the plant extracts

Plant material (reference compound)	Extract	MIC (mg/ml)			
		Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923	*Enterococcus faecalis* ATCC 29212
Aristolochia trilobata leaves	Hexane (2.3%)	2.5	2.5	0.31	NI
	Chloroform (1.7%)	NI	2.5	1.25	NI
	Methanol (9.8%)	2.5	NI	NI	NI
Aristolochia trilobata bark	Hexane (1.1%)	2.5	NI	0.625	NI
	Chloroform (1.1%)	NI	NI	NI	NI
	Methanol (7.5%)	2.5	2.5	NI	NI
Bursera simaruba bark	Hexane (0.7%)	2.5	2.5	NI	NI
	Chloroform (0.7%)	NI	NI	NI	NI
	Methanol (3.9%)	NI	NI	NI	NI
Guazuma ulmifolia bark	Hexane (0.2%)	2.5	NI	NI	NI
	Chloroform (0.7%)	NI	NI	NI	NI
	Methanol (4.7%)	NI	2.5	NI	NI
Hamelia patens leaves	Hexane (3.1%)	2.5	NI	NI	NI
	Chloroform (3.5%)	NI	NI	NI	NI
	Methanol (7.7%)	2.5	2.5	NI	NI
Syngonium podophyllum leaves	Hexane (1.7%)	2.5	2.5	NI	NI
	Chloroform (1.6%)	NI	NI	NI	NI
	Methanol (2.0%)	NI	NI	1.25	NI
Syngonium podophyllum bark	Hexane (0.6%)	NI	2.5	NI	NI
	Chloroform (0.5%)	NI	NI	NI	NI
	Methanol (1.3%)	2.5	NI	1.25	NI
TMP/SMZ[a]		0.002	0.032	0.001	0.010

NI: no inhibition; in parentheses the extraction yields (w/w) are reported.
[a] Trimethoprim/sulfamethoxazole.

the solvents were removed under vacuum at 30 °C until dry hexane, chloroform and methanol extracts were obtained. Extraction yields are reported in Table 1.

2.2. *Anti-bacterial activity*

Extracts were tested against the reference strains for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates, in duplicate, as reported by Koneman (1995) and Camporese (1997), and recommended by the National Committee for Clinical laboratory Standard (NCCLS, 2001).

The anti-bacterial activity of the extracts was tested against four aerobic reference bacterial strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. For susceptibility testing, in a first step 50 μl of Mueller Hinton broth were distributed from the second to the twelfth test tubes. Dry extracts were initially dissolved in 100 μl of dimethyl sulfoxide (DMSO) and then in Mueller Hinton Broth, to reach a final concentration of 10 mg/ml; 100 μl of these suspensions were added to the first test well of each microtiter line, and then 50 μl of scalar dilution were transferred from the second to the ninth well. The10th well was considered as growth control, since no extracts solutions were added. Then, 50 μl of a microbial suspension (10^5 colony forming units, CFU/ml), obtained from an overnight growth at 37 °C, were added to each well. The final concentration of the extracts adopted to evaluate the anti-bacterial activity was included from 5 mg/ml (first well) to 0.019 mg/ml (ninth well). Plates were incubated for 18 h at 37 °C and then they were examined from below with a reflective viewer and the lowest concentration of each extract showing growth was taken as its Minimal Inhibitory Concentration (MIC).

A blank control was taken using DMSO alone (100 μl/ml) added to the series of tubes and the MIC was evaluated as described above. No growth inhibition was observed at DMSO concentrations inferior or equal to 25 μg/ml.

The determination of the MICs of known anti-bacterial compounds (trimethoprim/sulfamethoxazole) for all the reference strains was simultaneously carried out. The experimental conditions, such as test medium and the number of bacteria determined by CFU/ml, were appropriate to reproduce inhibitory data of these known antibiotics, as recommended by NCCLS (NCCLS, 2001).

3. Results

Extracts were tested at various concentrations, ranging from 5 to 0.019 mg/ml, and the evaluated MIC values are

reported in Table 1. All the plants species showed activity to some extent against *Escherichia coli* and *Pseudomonas aeruginosa*, while *Aristolochia trilobata* leaves and bark, *Syngonium podophyllum* leaves and bark were active also against *Staphylococcus aureus*. Only *Enterococcus faecalis* was not inhibited by the studied herbal drugs. The reference anti-bacterial compounds, trimethoprim/sulfamethoxazole, exhibited the following MIC values: 0.032 mg/ml (*Pseudomonas aeruginosa*), 0.002 mg/ml (*Escherichia coli*), 0.010 mg/ml (*Enterococcus faecalis*) and 0.001 mg/ml (*Staphylococcus aureus*).

In particular, the Gram-positive *Staphylococcus aureus* was inhibited by the hexane extracts of *Aristolochia trilobata* leaves and bark, which showed the strongest activity (MIC = 0.31 and 0.625 mg/ml, respectively). A certain activity against this bacterium was shown also by the chloroform extract of *Aristolochia trilobata* leaves, which has a MIC value corresponding to 1.25 mg/ml and by the methanol extracts of *Syngonium podophyllum* leaves and bark, which showed a MIC value of 1.25 mg/ml (Table 1).

Escherichia coli was inhibited by almost all the hexane extracts (MIC = 2.5 mg/ml), except by that of *Syngonium podophyllum* bark. Furthermore, also the methanol extracts of *Aristolochia trilobata* leaves and bark, *Hamelia patens* leaves and *Syngonium podophyllum* bark showed a MIC of 2.5 mg/ml against *Escherichia coli* (Table 1).

Pseudomonas aeruginosa was inhibited by the hexane extracts of *Aristolochia trilobata* leaves, *Bursera simaruba* bark and *Syngonium podophyllum* leaves and bark at the same extent (MIC = 2.5 mg/ml). Also the chloroform extract of *Aristolochia trilobata* leaves inhibited the growth of this Gram-negative bacterium with the same potency of the methanol extracts of *Aristolochia trilobata* bark, *Guazuma ulmifolia* bark and *Hamelia patens* leaves (MIC = 2.5 mg/ml, Table 1).

4. Discussion

All extracts were able to inhibit the growth of one or more of the tested standard strains to a certain extent at 2.5 mg/ml that corresponds to a concentration of 0.25%. Several preparations for topical application are used as antibacterial agents at concentration of 0.25%. The highest activity was shown against the Gram-positive bacterium *Staphylococcus aureus*. These results are very interesting since this microorganism can be commonly involved in skin infections.

In particular, *Aristolochia trilobata* extracts showed an interesting anti-bacterial activity against *Staphylococcus aureus* with a MIC = 0.31 mg/ml for the hexane extract of the leaves and a MIC = 0.625 mg/ml for the hexane extract of the bark. This plant species is one of the most popular herbal remedies of Belize and it is used in traditional medicine for hangovers, flu, colds, amoebas, colitis and to clean the urinary tract (Balick and Arvigo, 1998). It seems that neither phytochemical nor biological studies have been carried out on this *Aristolochia* species: only its topical anti-inflammatory activity has been recently described (Sosa et al., 2002). However, plants belonging to the genus *Aristolochia*, were reported to contain aristolochic acids, which are known to be both mutagenic (Pfau et al., 1990; Bianucci et al., 1993; Goetzl and Schimmer, 1993; Pistelli et al., 1993) and carcinogenic (Xing et al., 1982; Schmeiser et al., 1989). The mutagenicity of these compounds could explain the mechanism of their anti-microbial action. In fact, these compounds isolated from *Aristolochia longa* (Hinou et al., 1990) inhibited *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. A similar compound form *Aristolochia contorta*, characterized as aristolactam *N*-(6′-*trans*-*p*-coumaroyl)-β-D-glucopyranoside, also showed anti-bacterial activity against Gram-positive bacteria (Lee and Han, 1992). It is noteworthy that the methanol but not hexane and chloroform extracts of *Aristolochia trilobata* leaves and bark possesses aristolochic acids and its derivatives. A number of pure aristolochic acids derivatives were isolated from methanol extract, after purification on Sephadex LH-20 column, followed by RP-HPLC and detected by TLC and NMR. Nevertheless, these compounds were not detected by TLC and NMR analyses of the fractions obtained, after chromatographic purification, of lipophilic extracts (data not shown). Therefore, compounds different from the mutagenic ones can be responsible for the interesting observed activity. Anyway, the traditional use of this plant species has to be discouraged, due to mutagenic and possibly human carcinogenic properties of aristolochic acids, present in the methanol extracts and probably also in the water preparations of aerial parts of the plant used in the folk medicine (Balick and Arvigo, 1998). Alternatively, lipophilic solvents such as sesame or peanut oil should be used to obtain an anti-microbial preparation avoided of possible side effects.

Concerning the other herbal drugs studied, *Bursera simaruba* bark, besides its use as wound healing, is widely used in folk medicine to alleviate the discomfort of insect bites, sunburn, rashes, skin sores; the decoction is taken as tea for internal infections, for urinary tract conditions, fevers, sun stroke, colds and flu (Balick and Arvigo, 1998). In our conditions, *Bursera simaruba* showed anti-bacterial activity against the Gram-negative tested strains, *Escherichia coli* and *Pseudomonas aeruginosa*. In a previous study (Cáceres et al., 1990) an ethanol extract (50% v/v) of *Bursera simaruba* bark did not show activity against *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, *Shighella dysenteriae* and *Shigella flexneri*. In another study (Bork et al., 1996), ethanol extract of *Bursera simaruba* showed activity against the non-pathogenic bacteria *Micrococcus luteus* (DSM 348), but not against *Escherichia coli* (DSM 1077) and *Staphylococcus aureus* (ATCC 25933). The few phytochemical studies available for this plant demonstrate the presence of some compounds as lignans (Ciccio and Rosales, 1995), triterpenes, lupeol, epilupeol, α–β amyrin,

epiglutinol (Peraza-Sanchez et al., 1995) and picropolygamain (Peraza-Sanchez and Pena-Rodriquez, 1992). Among these compounds, lupeol showed in vitro activity against both Gram-positive (*Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Proteus vulgaris* and *Pseudomonas pyocyanea*; Goyal and Rani, 1989). Anyway, lupeol was isolated from the plant resin and its presence in the bark was not reported until now.

For the first time, an anti-bacterial activity of *Guazuma ulmifolia* bark has been observed. Its hexane extract reduced the *Escherichia coli* growth, while an inhibition of *Pseudomonas aeruginosa* was obtained by the methanol extract. Although *Guazuma ulmifolia* bark is traditionally used for skin infections, sores and rashes (Balick and Arvigo, 1998), no studies are reported about anti-microbial activity of the bark of this species. On the contrary, its dried leaves tincture is active against *Bacillus subtilis* and *Escherichia coli* (Cáceres et al., 1987). Furthermore, the ethanol extract (50% v/v) of *Guazuma ulmifolia* leaf showed anti-bacterial activity against *Salmonella typhi* and *Shigella dysenteriae* (Cáceres et al., 1990). Some phytochemical studies on *Guazuma ulmifolia* demonstrate the presence of oil (Arriaga et al., 1996) and volatile constituents (Arriaga et al., 1997) in the leaves, and proanthocyanidin oligomers (Hoer et al., 1996) in the bark. Among them, proanthocyanidin oligomers could be present in the methanol extract. Therefore, it can be hypothesized a role of these compounds in the anti-bacterial activity of *Guazuma ulmifolia* bark methanol extract.

Hamelia patens leaves, flowers and stems are used in traditional medicine to treat skin problems, including sores, mycoses, insect bites, rashes, burns, itching and cuts (Balick and Arvigo, 1998). For stings from bees, wasps or "doctor fly", warmed leaf is applied as a poultice or the juice form crushed fresh leaves is rubbed on the sting (Balick and Arvigo, 1998). Our data partially confirm previous results about anti-microbial activity of this herbal drug. In fact, we found that both the hexane and the chloroform extracts of *Hamelia patens* inhibited the growth of *Escherichia coli*; in addition its methanol extract inhibited also *Pseudomonas aeruginosa*, but not *Staphylococcus aureus*. In a previous study, an ethanol extract of *Hamelia patens* showed activity against *Staphylococcus aureus*, while a water extract was active against *Escherichia coli*, *Salmonella typhi*, *Sarcina lutea*, *Serratia marcescens* and *Shigella flexneri* (Jimènez Misas et al., 1979). The phytochemical studies on this species revealed the presence of some oxindole alkaloids (Borges del Castillo et al., 1982; Martinez et al., 1996), a flavanone glycoside (2′,5,5′,7-tetrahydroxyflavanone 7-rutinoside), narirutin, rosmarinic acid (Aquino et al., 1990), flavonoids (Tiwari et al., 1978) and isopteropodine (Ripperger, 1977). A recent study demonstrated an anti-bacterial activity of rosmarinic acid (Li et al., 2000) against *Escherichia coli* and *Staphylococcus aureus*. This compound can be also present in the methanol extract of *Hamelia patens* leaves and could partially contribute also for the activity reported in this study.

Syngonium podophyllum leaves are used in Central American traditional medicine for skin conditions, sores, dry skin, itching, rashes and bruises, while leaf tincture is used for rheumatism, arthritis and general pains and swellings (Balick and Arvigo, 1998). The methanol extracts of *Syngonium podophyllum* leaves and bark showed an activity against *Staphylococcus aureus*. This is the first report about anti-bacterial activity of this species, for which only limited biological studies and phytochemical research have been carried out until now.

In conclusion, the obtained results confirm the presence of anti-bacterial principles in all of the examined herbal drugs: in particular, an anti-microbial activity has been reported for the first time for two of the studied species (*Aristolochia trilobata* and *Syngonium podophyllum*). Recently, some of these herbal drugs showed also a topical anti-inflammatory activity that, together with the anti-bacterial properties, supports their traditional use as wound healing in Central America. Furthermore, beside the confirmation of the popular use, the obtained results demonstrate that these herbal drugs could represent a new source of anti-microbial agents, less expensive than the imported drugs. In this respect, it will be very interesting to carry out a bioassay-oriented fractionation of the active extracts to isolate the pure compounds responsible for the observed activities.

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