Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Ethnopharmacology 99 (2005) 309–312

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Short communication

Antibacterial screening of some Peruvian medicinal plants used in Callería District

P. Kloucek[a], Z. Polesny[a], B. Svobodova[b], E. Vlkova[c], L. Kokoska[a,*]

[a] Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Agriculture Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic
[b] Department of Biochemistry, Faculty of Science, Charles University in Prague, Albertov 6, Prague 128 43, Czech Republic
[c] Department of Microbiology, Nutrition and Dietetics, Faculty of Agronomy, Czech University of Agriculture Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic

Received 22 October 2004; received in revised form 22 December 2004; accepted 28 January 2005
Available online 8 April 2005

Abstract

Nine ethanol extracts of *Brunfelsia grandiflora* (Solanaceae), *Caesalpinia spinosa* (Caesalpiniaceae), *Dracontium loretense* (Araceae), *Equisetum giganteum* (Equisetaceae), *Maytenus macrocarpa* (Celastraceae), *Phyllanthus amarus* (Euphorbiaceae), *Piper aduncum* (Piperaceae), *Terminalia catappa* (Combretaceae), and *Uncaria tomentosa* (Rubiaceae), medicinal plants traditionally used in Callería District for treating conditions likely to be associated with microorganisms, were screened for antimicrobial activity against nine bacterial strains using the broth microdilution method. Among the plants tested, *Phyllanthus amarus* and *Terminalia catappa* showed the most promising antibacterial properties, inhibiting all of the strains tested with minimum inhibitory concentrations (MICs) ranging from 0.25 to 16 mg/ml. The extract from aerial part of *Piper aduncum* was significantly more active against Gram-positive (MICs ranging from 1 to 2 mg/ml) than against Gram-negative bacteria (MICs >16 mg/ml).

Keywords: Antibacterial activity; Crude extracts; Peruvian medicinal plants; *Phyllanthus amarus*; *Terminalia catappa*

1. Introduction

Callería District, part of the Coronel Portillo Province in the Ucayali Department, lies in the lowlands of Peruvian Amazonia. Pucallpa, its administrative centre and also the capital of the Ucayali Department, is situated on the banks of the river Ucayali, a principal tributary of the Amazon. The largest indigenous ethno-linguistic group living in this region is the Shipibo-Conibo group. The Ucayali department, formerly very isolated from other parts of Peru, is now one of the fastest developing Peruvian regions, mainly due to extensive logging. However, timber production together with shifting agriculture has a devastating effect on natural ecosystems and can lead to high genetic erosion. Thanks to the indigenous inhabitants, who still largely depend on natural resources, the important ethnomedicinal knowledge of this region survives. Nevertheless, it should be verified and preserved by modern scientific methods.

The phytochemical research based on ethnopharmacological informations is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. In Peru about 20,000 plant species or 8% of the total number of plants in the world can be found. Most of them are native or grow in the Peruvian Amazonia. However, probably less than 1% has been studied for their chemical composition and medicinal use (Desmarchelier and Schaus, 2000). In this study, we chose nine promising medicinal plants used traditionally by local inhabitants of Callería District for treating conditions likely to be associated with microorganisms, and evaluated them for potential antibacterial activity, in order to confirm their

* Corresponding author. Tel.: +420 224382180; fax: +420 234381829.
E-mail address: kokoska@itsz.czu.cz (L. Kokoska).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.01.062

Table 1
Ethnobotanical data on medicinal plants

Species (family) and voucher specimen number	Common name	Part tested	Ethnomedicinal uses
Brunfelsia grandiflora D. Don (Solanaceae) POL 0001	Chirisanango	Roots	Diuretic, febrifuge, antirheumatic, antisyphilitic, against yellow fever (Schultes and Raffauaf, 1990; Plowman, 1977; Desmarchelier and Schaus, 2000)
Caesalpinia spinosa (Molina) Kuntze (Caesalpiniaceae) POL 0002	Tara	Pods	Eyewash (Duke and Reed, 1981)
Dracontium loretense K. Krause (Araceae) POL 0003	Sacha jergón	Tubers	Treatment of snakebites (Desmarchelier and Schaus, 2000), antidiarrheic (Schultes and Raffauaf, 1990)
Equisetum giganteum L. (Equisetaceae) POL 0004	Cola de caballo	Aerial part	Astringent, antidiarrheic, diuretic, emmenagogue, wound healing (Desmarchelier and Schaus, 2000)
Maytenus macrocarpa Briq. (Celastraceae) POL 0005	Chuchuhuasi	Bark	Anti-inflammatory, antirheumatic, tonic, aphrodisiac (Desmarchelier and Schaus, 2000)
Phyllanthus amaraus Schum. et Thonn. (Euphorbiaceae) POL 0007	Chanca piedra	Aerial part	Diuretic, sedative, astringent, tonic, antidiabetic, antihemorrhagic, against jaundice and kidney diseases (Schultes and Raffauaf, 1990; Desmarchelier and Schaus, 2000)
Piper aduncum L. (Piperaceae) POL 0006	Matico	Aerial part	Antiseptic, antidiarrheic, tonic, astringent, antirheumatic, styptic (Schultes and Raffauaf, 1990; Desmarchelier and Schaus, 2000)
Terminalia catappa L. (Combretaceae) POL 0008	Almendra	Leaves	Treatment of bilious fevers and dysentery (Schultes and Raffauaf, 1990)
Uncaria tomentosa DC. (Rubiaceae) POL 0009	Uña de gato	Bark	Anti-inflammatory, antidiabetic, anticancer (Desmarchelier and Schaus, 2000)

popular use and to detect new sources of antibacterial agents.

2. Materials and methods

2.1. Plant material and extract preparation

After consultations with village elders and herbalists and comparing their information with literature, we chose nine plants that offered good prospects (Table 1). Samples for this study were obtained from marketplace vendors and herbalists in Pucallpa, and authenticated by Z. Polesny. Voucher specimens are deposited at the Institute of Tropics and Subtropics, Czech University of Agriculture Prague.

Air dried plant material (15 g of each species) was finely ground and macerated at room temperature in 80% ethanol for 5 days. The extract was subsequently filtered and concentrated in vacuo at 40 °C. The residue was dissolved in 10% (v/v) solution of dimethylsulfoxide (DMSO) in Tris buffer saline (TBS) of pH 7.6 (Sigma, USA) to create a concentration of 32 mg/ml of stock solution.

2.2. Microorganisms

The following strains of bacteria were used: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Bacteroides fragilis* ATCC 25285, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and *Streptococcus pyogenes* ATCC 19615.

Streptococci were grown and tested in brain–heart infusion broth, *Bacteroides fragilit* in Wilkins-Chalgren anaerobe broth under anaerobic conditions using Anaerobic Jar HP11 (Oxoid, UK). Other microorganisms were grown and tested in Mueller–Hinton broth.

All microbial strains and cultivation media were purchased from Oxoid (UK). Ciprofloxacin (Sigma, USA) was checked as positive control (Table 2).

2.3. Antibacterial assay

In vitro antimicrobial activity was determined by the broth microdilution method (Jorgensen et al., 1999) using 96-well microtitre plates. Two-fold dilutions (six) of each extract were carried out, starting from a concentration of 16 mg/ml. Each well was inoculated with 5 μl of bacterial suspension at a density of 10^7 CFU/ml and incubated at 37 °C for 24 h. The growth of microorganisms was observed as turbidity determined by the UV–vis spectrometer Helios ε (Spectronic Unicam, UK) at 600 nm. The MIC was determined as the lowest dilution which completely prevented microbial growth. The solution of DMSO (5%, v/v) in TBS served as the negative control. All samples were tested in triplicate.

3. Results

Table 1 shows the botanical name, local name, voucher specimen number, plant part investigated and popular uses of the selected plant species. Table 2 gives a summary of the investigated species, the percentage yield and the obtained MIC values. With exception of *Maytenus macrocarpa*, all plants tested possessed antibacterial activity against at least four bacterial strains (*Bacillus cereus*, *Enterococcus faecalis*, and both staphylococci) at the concentrations of 16 mg/ml or below. Extracts from aerial part of *Phyllanthus amarus* and from leaves of *Terminalia catappa* showed the broadest spec-

Table 2
Minimum inhibitory concentrations (mg/ml) of ethanol extracts of nine Peruvian medicinal plants

Species, reference compound	Extract yield (%)	Gram-positive						Gram-negative		
		B.c.	*B.s.*	*E.f.*	*S.a.*	*S.e.*	*S.p.*	*B.f.*	*E.c.*	*P.a.*
Brunfelsia grandiflora	13.53	4	16	8	4	4	NA	16	NA	16
Caesalpinia spinosa	48.67	8	16	0.5	16	16	–	16	NA	NA
Dracontium loretense	7.87	8	NA	8	8	16	NA	NA	NA	NA
Equisetum giganteum	7.33	8	16	8	8	16	4	8	NA	NA
Maytenus macrocarpa	11.93	NA	NA	NA	NA	NA	–	NA	NA	NA
Piper aduncum	6.07	1	1	2	1	2	2	NA	NA	NA
Phyllanthus amarus	13.13	16	1	0.25	4	1	4	4	16	8
Terminalia catappa	14.53	2	4	8	1	0.25	16	16	8	4
Uncaria tomentosa	6.67	1	1	0.25	1	1	NA	NA	8	NA
CIP (μg/ml)[a]		1	2	1	0.5	1	1	2	0.015	0.25

–: Not performed; NA: not active (>16). B.c., *Bacillus cereus*; B.s., *Bacillus subtilis*; B.f., *Bacteroides fragilis*; E.f., *Enterococcus faecalis*; E.c., *Escherichia coli*; P.a., *Pseudomonas aeruginosa*; S.a., *Staphylococcus aureus*; S.e., *Staphylococcus epidermidis*; S.p., *Streptococcus pyogenes*.
[a] CIP, ciprofloxacin.

trum of action against bacteria, inhibiting all of the strains tested with minimum inhibitory concentrations (MICs) ranging from 0.25 to 16 mg/ml. The strongest activity (MIC 0.25 mg/ml) was shown by *Phyllanthus amarus* and *Uncaria tomentosa* against *Enterococcus faecalis*, and *Terminalia catappa* against *Staphylococcus epidermidis*. The extract from aerial part of *Piper aduncum* was significantly more active against Gram-positive (MICs ranging from 1 to 2 mg/ml) than against Gram-negative bacteria (MICs >16 mg/ml). Good activity was observed also in the *Uncaria tomentosa* extract, which inhibited six bacteria and five of them with MICs from 0.25 to 1 mg/ml. Other extracts showed only slight inhibition of tested microorganisms.

Of the bacteria tested, *Escherichia coli* proved to be the most difficult to inhibit with only slight susceptibility to the *Phyllanthus amarus*, *Terminalia catappa* and *Uncaria tomentosa* extracts (MICs ranging from 8 to 16 mg/ml). Gram-negative bacteria were generally less susceptible than Gram-positive. The most sensitive bacterium was *Enterococcus faecalis*, which was inhibited by all tested species except *Maytenus macrocarpa* with MICs ranging from 0.25 to 8 mg/ml.

No growth inhibition was observed in the negative control.

4. Discussion and conclusions

The use of these plants in Peruvian Amazon folk medicinal remedies for treating various health problems has already been reported (Schultes and Raffauaf, 1990; Desmarchelier and Schaus, 2000); with the most frequent medicinal uses of the investigated plants being antirheumatic, antidiarrheic, astringent, diuretic and tonic (3 plants), antidiabetic and antiinflammatory (2 plants). However, apart from *Phyllanthus amarus*, *Piper aduncum* and *Terminalia catappa*, no scientific information concerning the antibacterial properties of these plants has been reported.

Crude extract of *Phyllanthus amarus* collected in India possessed antibacterial activity against *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella paratyphi* and *Staphylococcus aureus* (Srinivasan et al., 2001). Among compounds isolated from *Phyllanthus amarus*, tannins corilagin, geraniin and gallic acid (Foo, 1993) showed in vitro activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Adesina et al., 2000; Gohar et al., 2003).

Antimicrobial properties of *Piper aduncum* have been reported (Lentz et al., 1998; Lemos et al., 2000). Benzoic acid and benzene derivates, dihydrochalcones and chromenes isolated from its leaves were active against a variety of microorganisms including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Cryptococcus neoformans*, *Mycobacteria intracellulare*, *Micrococcus luteus* and *Pseudomonas aeruginosa* (Nair and Burke, 1990; Orjala et al., 1993; Orjala et al., 1994; Okunade et al., 1997). The chemical composition of essential oil and its antibacterial properties have also been described (Tirillini et al., 1996; Maia et al., 1998; Pino et al., 2004).

High antifungal but no antibacterial activity of methanol and methylene chloride extracts from *Terminalia catappa* aerial part (Goun et al., 2003) has been reported. On the other hand different authors (Pawar and Pal, 2002) investigated roots of *Terminalia catappa* and detected good antimicrobial activity of chloroform and methanol extracts against *Escherichia coli* and *Staphylococcus aureus*, which supports our results. The phytochemical studies on *Terminalia catappa* bark and leaves demonstrate the presence of tannins and flavonoid glycosides (Lin and Hsu, 1999; Lin et al., 2000). Among them, gallic acid, corilagin, ellagic acid and rutin showed in vitro antibacterial activity (Adesina et al., 2000; Basile et al., 2000; Thiem and Goslinska, 2004).

No previous reports on the antibacterial activity of the other species could be found in literature. Flavonol glycosides were isolated from aerial parts of *Brunfelsia grandiflora* (Brunner et al., 2000) and some of these structures are known to be responsible for antibacterial activity of higher plants (Yadava and Reddy, 1998; Cui et al., 2003). Pods of *Caesalpinia spinosa* contain up to 25% of gallic acid (Galvez et al., 1997) and previously this tannin isolated from *Acalypha wilkesiana*, *Acalypha hispida* and *Rubus ulmifolius* showed in vitro antimicrobial activity when tested against *B. cereus*, *Staphylococcus aureus*, and *Escherichia coli* (Adesina et al., 2000; Panizzi et al., 2002). Among the classes of compounds identified in *Uncaria tomentosa* (Montoro et al., 2004), antibacterial activity is attributed to some triterpenes (Akbar and Malik, 2002).

From nine plants, eight showed antimicrobial properties (89%), which confirm their popular use and justify the ethnobotanical approach in the search for novel biologically active compounds. For five of them this is the first report of such activity. Among the medicinal plants tested in this work, *Phyllanthus amarus* and *Terminalia catappa* showed the most promising antibacterial properties, indicating the potential for discovery of antibacterial principles. We assume that some of the previously isolated compounds could be present in the extracts investigated and could partially contribute to antimicrobial activity reported in this study. However, further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these species.

Acknowledgements

This work was supported by projects 80/03-06/MZe/B and GACR 523/03/H076.

References

Adesina, S.K., Idowu, O., Ogundaini, A.O., Oladimeji, H., Olugbade, T.A., Onawunmi, G.O., Pais, M., 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytotherapy Research 14, 371–374.

Akbar, E., Malik, A., 2002. Antimicrobial triterpenes from *Debregeasia salicifolia*. Natural Product Letters 16, 339–344.

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., Cobianchi, R.C., Vuotto, M.L., Ferrara, L., 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia 71, 110–116.

Brunner, G., Burger, U., Castioni, P., Kapetanidis, I., Christen, P., 2000. A novel acylated flavonol glycoside isolated from *Brunfelsia grandiflora* ssp. *grandiflora*. Structure elucidation by gradient accelerated NMR spectroscopy at 14T. Phytochemical Analysis 11, 29–33.

Cui, G.Y., Liu, J.Y., Tan, R.X., 2003. A new antimicrobial flavonol glycoside from *Alchornea davidii*. Chinese Chemical Letters 14, 179–180.

Desmarchelier, C., Schaus, F.W., 2000. Sixty medicinal plants from the Peruvian Amazon, 1st ed. Lima, Peru, pp. 81–243 (privately published).

Duke, J.A., Reed, C.F., 1981. *Caesalpinia spinosa* (Mol.) Ktz. In: Duke, J.A. (Ed.), Handbook of Legumes of World Economic Importance. Plenum Press, New York, USA, pp. 32–33.

Foo, L.Y., 1993. Amariin, a di-dehydrohexahydroxydiphenoyl hydrolyzable tannin from *Phyllanthus amarus*. Phytochemistry 33, 487–491.

Galvez, J.M.G., Riedl, B., Conner, A.H., 1997. Analytical studies on tara tannins. Holzforschung 51, 235–243.

Gohar, A.A., Lahloub, M.F., Niwa, M., 2003. Antibacterial polyphenol from *Erodium glaucophyllum*. Zeitschrift fur Naturforschung 58, 670–674.

Goun, E., Cunningham, G., Chu, D., Nguyen, C., Miles, D., 2003. Antibacterial and antifungal activity of Indonesian ethnomedical plants. Fitoterapia 74, 592–596.

Jorgensen, J.H., Turnidge, J.D., Washington, J.A., 1999. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H. (Eds.), Manual of Clinical Microbiology, 7th ed. ASM Press, Washington, DC, pp. 1526–1543.

Lemos, G.C.S., Oliveira, L.O., Eberli, B.B., Motta, O.V., Folly, M.M., 2000. Bactericidal activity of macela (*Achyrocline satureioides* (*Lam.*) DC.) and jaborandi-falso (*Piper aduncum* L.) against strains of *Staphylococcus aureus* isolated from subclinical bovine mastitis. Revista Brasileira de Plantas Medicinais 3, 67–72.

Lentz, D.L., Clark, A.M., Hufford, C.D., Meurer-Grimes, B., Passreiter, C.M., Cordero, J., Ibrahimi, O., Okunade, A.L., 1998. Antimicrobial properties of Honduran medicinal plants. Journal of Ethnopharmacology 63, 253–263.

Lin, T.C., Hsu, F.L., 1999. Tannin and related compounds from *Terminalia catappa* and *Terminalia parviflora*. Journal of the Chinese Chemical Society 46, 613–618.

Lin, Y.L., Kuo, Y.H., Shiao, M.S., Chen, C.C., Ou, J.C., 2000. Flavonoid glycosides from *Terminalia catappa* L. Journal of the Chinese Chemical Society 47, 253–256.

Maia, J.G.S., Zohhbi, M.D.B., Andrade, E.H.A., Santos, A.S., da Silva, M.H.L., Luz, A.I.R., Bastos, C.N., 1998. Constituents of the essential oil of *Piper aduncum* L. growing wild in the Amazon region. Flavour and Fragrance Journal 13, 269–272.

Montoro, P., Carbone, V., Quiroz, J.D., De Simone, F., Pizza, C., 2004. Identification and quantification of components in extracts of *Uncaria tomentosa* by HPLC-ES/MS. Phytochemical Analysis 15, 55–64.

Nair, M.G., Burke, B.A., 1990. Antimicrobial *Piper* metabolite and related compounds. Journal of Agricultural and Food Chemistry 38, 1093–1096.

Okunade, A.L., Hufford, C.D., Clark, A.M., Lentz, D., 1997. Antimicrobial properties of the constituents of *Piper aduncum*. Phytotherapy Research 11, 142–144.

Orjala, J., Erdelmeier, C.A.J., Wright, A.D., Rali, T., Sticher, O., 1993. 5 new prenylated *p*-hydroxybenzoic acid-derivatives with antimicrobial and molluscicidal activity from *Piper aduncum* leaves. Planta Medica 59, 546–551.

Orjala, J., Wright, A.D., Behrends, H., Folkers, G., Sticher, O., Ruegger, H., Rali, T., 1994. Cytotoxic and antibacterial dihydrochalcones from *Piper Aduncum*. Journal of Natural Products 57, 18–26.

Panizzi, L., Caponi, C., Catalano, S., Cioni, P.L., Morelli, I., 2002. In vitro antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius*. Journal of Ethnopharmacology 79, 165–168.

Pawar, S.P., Pal, S.C., 2002. Antimicrobial activity of extracts of *Terminalia catappa* root. Indian Journal of Medical Sciences 56, 276–278.

Pino, J.A., Marbot, R., Bello, A., Urquiola, A., 2004. Essential oils of *Piper peltata* (L.) Miq. and *Piper aduncum* L. from Cuba. Journal of Essential Oil Research 16, 124–126.

Plowman, T., 1977. Brunfelsia in ethnomedicine. Botanical museum leaflets 25, 289–320.

Schultes, R.E., Raffauaf, R.F., 1990. The healing forest. Dioscorides press, Portland, USA, p. 484.

Srinivasan, D., Nathan, S., Suresh, T., Perumalsamy, P.L., 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology 74, 217–220.

Thiem, B., Goslinska, O., 2004. Antimicrobial activity of *Rubus chamaemorus* leaves. Fitoterapia 75, 93–95.

Tirillini, B., Velasquez, E.R., Pellegrino, R., 1996. Chemical composition and antimicrobial activity of essential oil of *Piper angustifolium*. Planta Medica 62, 372–373.

Yadava, R.N., Reddy, K.I.S., 1998. A new big-active flavonol glycoside from the stems of *Butea superba* Roxb. Journal of Asian Natural Products Research 1, 139–145.