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Journal of Ethnopharmacology 96 (2005) 195–200
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Screening of some New Caledonian and Vanuatu medicinal plants for antimycobacterial activity

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Received 17 February 2004; received in revised form 18 August 2004; accepted 2 September 2004
Available online 27 October 2004

Abstract

Twenty plants, belonging to sixteen families, used in traditional New Caledonian and Vanuatu medicine for treatment of symptoms potentially related to tuberculosis (cough, fever or inflammation) were screened for antimycobacterial activity. We also screened an original endemic plant, *Amborella trichopoda*, only member of the monogeneric family Amborellaceae and considered the most primitive living angiosperm. In total, 55 extracts were evaluated for inhibitory activity against *Mycobacterium bovis* BCG strain at a concentration of 100 μg/ml. Methanolic and dichloromethane extracts of *Amborella trichopoda*, *Codiaeum peltatum*, *Myristica fatua*, and essential oils *Myoporum crassifolium* showed an activity at this concentration. Methanolic extract of *Amborella trichopoda* fruits presented a significant activity with a minimal inhibitory concentration included between 1 and 2.5 μg/ml. In the same conditions, this activity was comparable with those of the reference drugs pyrazynamide and ethambutol, at 20 and 2.5 μg/ml, respectively.

Keywords: New Caledonian and Vanuatu medicinal plants; *Amborella trichopoda* tuberculosis; *Mycobacterium bovis*

1. Introduction

The resurgence of tuberculosis is one of the most serious public health challenge of the 21st century. Despite continued control efforts, tuberculosis (TB) remains a leading cause of illness and death worldwide (WHO, 2001). It is estimated that over eight million people contract tuberculosis each year, and approximately two to three million people die from this disease (Dye et al., 1999; Zumla et al., 1999). The recent increasing of TB is associated with the emergence of the human immunodeficiency virus (HIV) and the rapid spread of multidrug resistant TB strains. Current treatment of TB requires a patient takes at least three or four antituberculosis drugs. The leading therapy associates isionazid and rifampicin and may be supplemented with pyrazinamide and ethambutol when a resistant strain is suspected (Zumla and Grange, 2001). The rapid extension of TB has accelerated the need for more efficient drugs to combat this infection.

A recent report (WHO, 2001) states that in the Western Pacific area 1.5 million cases are notified each year. In New Caledonia, the incidence of TB dropped in 12 years to 48 for 100,000, which stays relatively important, the mean incidence being 62.5 for 100,000 in the 12 years to year 2000, with extremes of 11.6 for 100,000 by European people and 84 for 100,000 by Melanesian people; on a total of 1427 cases of different forms of TB, 12 fatal cases occured in 12

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doi:10.1016/j.jep.2004.09.008

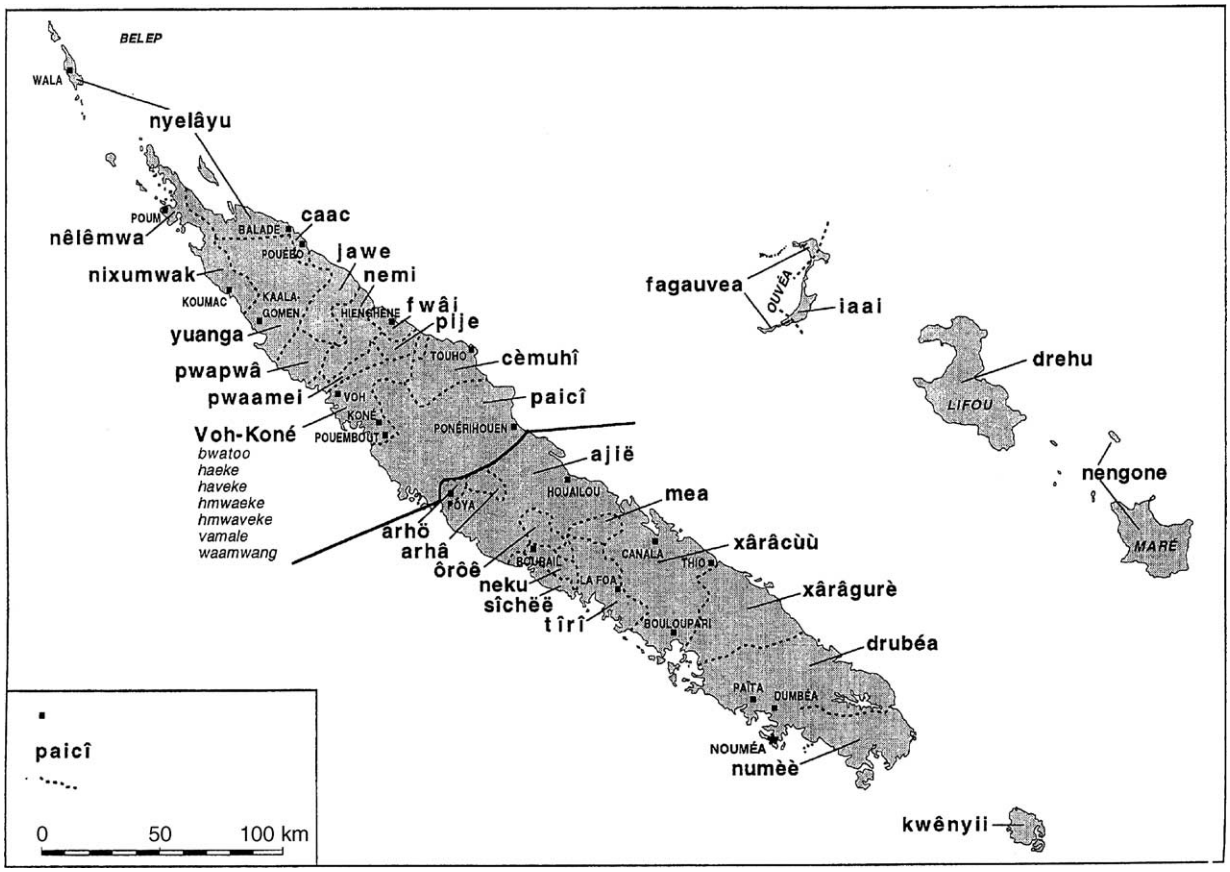

Fig. 1. Languages from New Caledonia and Loyalty Islands (adapted from Bril, 2000).

years (DASS NC, 2000). Traditional remedies are still used in New Caledonia and widely in Vanuatu; they can contain natural antimicrobials which are ingested by patients when New Caledonian healers recommend medicinal plants in curing TB-related symptoms such as blood in the sputum, cough or fever.

More generally, medicinal plants remain an important resource to find original active drugs or new therapeutic agents. Over 350 natural products (Newton et al., 2000) have been evaluated for their antimycobacterial activities. Some compounds, such as alkaloids (Houghton et al., 1999; Newton et al., 2002), chalcones, flavonoids (Lin et al., 2002) or terpenoids (Cantrell et al., 2001) have demonstrated in vitro antimycobacterial activity.

However, we have initiated investigations to find new natural active compounds against tuberculosis. In a preliminary screening we selected 20 plants (one species with two botanical varieties) medicinally used in traditional treatments of TB-related symptoms, or particularly interesting species like the endemic plant *Amborella trichopoda* Baill. (Amborellaceae), considered to be the most primitive angiosperm, on the base of phylogenetic data (Zanis et al., 2002).

In this study, we used a simple in vitro screening assay using a 96-well microplate dilution method. The plant extracts were screened against slow growing and non-pathogenic mycobacteria, *Mycobacterium bovis* BCG strain.

2. Materials and methods

2.1. Plant material

Twenty-two plants from 16 families, native from New Caledonia, were selected on the basis of ethnopharmacological information (especially treatment of some tuberculosis related symptoms: blood in the sputum, fever, cough) or on their potential antiseptic or antiinflammatory properties, as presumed following local medicinal knowledge (see Fig. 1, the ethnological map of New Caledonia and Loyalty Islands). They were identified at the SNT&ST and at the Botany Laboratories, Institut de recherche pour le développement (IRD), Nouméa, New Caledonia. Voucher specimens are deposited in the Herbarium of IRD Centre, Nouméa. Plant materials were oven-dried at 40 °C and then ground.

2.2. Preparation of plant extracts

2.2.1. Preparation of crude ethanolic extracts

Dried powdered material (50 g) was extracted by maceration in 250 ml of ethanol (80%) for 3 h under shaking at ambient temperature. The extract was filtered and the residual powder was extracted again by a second maceration in 100 ml of ethanol (80%) for 1 h under shaking and then filtered. The

Table 1
Plants studied and their traditional use Melanesian languages: orthography as in Bril (2000) (see Fig. 1)

Plant species (family) statute	Voucher specimen no.	Vernacular name (Melanesian language, or 'ncf', New Caledonia French)	Part(s) used and (use modality)	Traditional use in medicine (possibly TB, inflammation, infections) or other potential
Acronychia laevis Forst. and G. Forst. (Rutaceae) native	JW-RG14	*gerit, görit* (jawe), *bouï* (xârâcùù), *pô* (drubéa)	Leaves, maceration (per os)	*All diseases*, to provoke menstruations *lepra*, herpes, *fever* all diseases *post partum* cleaning purge, depuration 'blood cleaning' epilepsy crisis *sprains* (hemostase and resolution) cicatrisation against vomiting
		gerit (nemi)	Bark, lixiviation (per os)	
		bwèit (cèmuhî)	Young shoots (per os)	
		bui (ajië)	Bark, maceration (per os)	
		pwë, *poé*, poü (kwênyii), *poë* (mea)	Leaves, maceration (per os)	
		boï (sîchëë)	Leaves, decoction (bath)	
		pojè (drehu)	Leaves, maceration (per os, bath)	
		bolè (nengone)	Wood or leaves decoction (per os) Juice of leaves, chewed	
Amborella trichopoda Baill. (Amborellaceae) endemic	PC21-1-03	No vernacular name collected	No use collected	(Endemic and very original species)
Babingtonia leratii (Schl.) A.R. Bean (Myrtaceae) endemic	JWRG5	*fausse bruyère* (ncf)	A handful of flowering branches, decoction (per os)	*Cystitis*
		noku in (kwênyii)	Leaves, decoction (per os)	*Rhumatisms*
Cerberiopsis candelabra Vieill. ex Pancher and Sebert var. *vexillaria* (Däniker) P. Boiteau (Apocynaceae) endemic		*candélabre* (ncf), *kasia* (drehu)	Latex	*Violent poison*
Codiaeum peltatum (Labill. P.S. Green (Euphorbiaceae) native	JW-RG23	*croton* (ncf)		*Tuberculosis*
Crossostylis multiflora Brongn. and Gris ex Pancher and Sebert (Rhizophoraceae) endemic	JW-RG20	*hêtre noueux*, *chêne gris de Farino* (ncf)	Bark, decoction	Ulcerations (external), *yaws*
		opwäro (paicî)		
Cupaniopsis glomeriflora Radlk (Sapindaceae) endemic		*chêne blanc* (ncf), *sij* (drehu)	Leaflets, mastication (per os)	*Fatigue* headache
			Leaves (per os, baths, instillations)	
Drosera neocaledonica Raym. Hamet (Droseraceae) endemic	PC3194	*gobe mouche* (ncf)	Stems without flowers, maceration whole plant, maceration (per os)	*Chest pains*, *tuberculosis*, stomachic use
	PC3239			
Erythrina variegata var. *fastigiata* (Guillaumin) combined (Fabaceae) (endemic variety)	JWRG8	*cère jié* [cére-male] (numéé), '*peuplier canaque mâle*' (transposition of local conception of duality in the species to ncf)	Bark, maceration (per os)	Ciguatera
			Bark, infusion (per os)	Fortifying remedy
		kopwa (xârâguré, region of Bangou, old contacts with drubéa)	Decoction of leaves (baths)	Ciguatera
		näru (paicî), *peuplier* (ncf)	Bark, maceration (per os)	Fortifying remedy syphilis, diabetes
		dalep dahwapwê [=fir-*dalep*] (jawe)	Bark, maceration (per os)	Ache all over
		amî (iaai), *ngetae* (fagauvea)	Tepid maceration (per os)	*Fever*
		doru (ajië)	Bark, maceration (per os)	Ciguatera
		nëru pao [=shadow-*nëru*] (paicî)	Bark, maceration (per os)	Urinary infection
Fontainea pancheri (Baill.) Heckel (Euphorbiaceae) native		No vernacular name collected		*Ichtyotoxic*
Fagraea berteriana Benth. (Loganiaceae) native		*bois pétrole*, *bois tabou* (ncf), *buac* (nyelâyu)	Leaves, bark	*All diseases*, emollient rhumatisms, purge

Table 1 (*Continued*).

Plant species (family) statute	Voucher specimen no.	Vernacular name (Melanesian language, or 'ncf', New Caledonia French)	Part(s) used and (use modality)	Traditional use in medicine (possibly TB, inflammation, infections) or other potential
		guèc (jawe), *guèc* (nemi), *buè* (xârâgurè), *pue* or *bwe* (numèè)	Infusion (per os)	
		mêdügö (paicî), *biyoâ* (ajië)	Bark, maceration (per os)	
		mindugo (arhö)	Leaves, heated, extraction of juice leaves, heated	*Otitis*, convulsions, excitation
		pohè (tîrî)	Leaves, heated roots maceration (per os)	*Irritations* caused by the latex of *Semecarpus* spp. mild aborfacient sexual diseases strong purgation, *sprains*, stops menstruations anticonceptional use for a good growth of babies "*white tongue*" (probably candidosis) constipation, crisis of epilepsy *cicatrisation*
		potr (drehu)	Bark, maceration (per os)	
		puja puja (nengone)	Fruits leaves, infusion (per os)	
Gardenia urvillei Montrouz. (Rubiaceae) endemic	JW77	*tiaré des forêts sèches* (ncf), *peiokwé* (ajië)	Bark maceration (per os)	
Glochidion billardieri Baill. (Euphorbiaceae) endemic	JWRG3	*omwarrà* (ôrôê)	Leaves, mastication (per os)	
		hmana (drehu)	Leaves, mastication (per os)	
		hmana (iaai)	Leaves, mastication (per os)	Rhumatisms
Melodinus scandens J.R. Forster and G. Forster (Apocynaceae) native	JWRG6	*girawa* (nemi)	Leaves, decoctin, massage leaves, mastication leaves, decotion complex preparation	Sprains *pulmonary congestion cough* drugs for babies *wood essential oil*
		wa rui (ajië), *kuimuea* (xârâcùù) *nyihlë*, *hnyimesa*, *hnimösa* (drehu)		
Myoporum crassifolium Forster and G. Forster (Myoporaceae) native	DN93	*ndji* (kwênyii, and numèè, partly, region of Goro)		
Myristica fatua Houtt. (Myristicaceae) native from Vanuatu absent in NC	cf PC1046	*nandai* in bislama pidgin, *wild nutmeg* in Vanuatu english, Vanuatu vernacular names in Wheatley 1992; species native to the flora of Vanuatu	Sap fruit leaves	Uses in Vanuatu (Lefeuvre, 1996) *yaws*? dental protection (actinomycetes?) childbirth
Piper methysticum G. Forst. (Piperaceae) native to Vanuatu introduced in NC	Commercial sample from Vanuatu	'kava' in bislama pidgin, Vanuatu French, ncf, English	Root (preparations per os)	Potential of kavalactones, following some Vanuatu traditional uses: urogenital diseases *rheumatisms* gastrointestinal problems *respiratory irritations pulmonary pains*
		Vernacular Vanuatu names: Lebot and Cabalion (1986); species native to the flora of Vanuatu	Leaves (preparations for skin application)	
Santalum austrocaledonicum Vieill. (Santalaceae) native	JWRG7	*santal* (ncf)	Leaves, infusion (per os)	Ciguatera *use as a vulnerary cough*, influenza
		tapaka (numèè, partly: Ile Ouen)	Leaves, decotion	
		tepagaï (numèè)	Leaves, maceration or mastication	
		tapakas, *tapakai*, *trapakae* (drehu)	Leaves, decoction	Blennoragy
		wahàta (iaai)		
		wekes(*i*) (nengone)		
Smilax orbiculata Vieil. ex A.DC (Smilacaceae) endemic	JWRG1	*salsepareille* (ncf)		

Table 1 (*Continued*).

Plant species (family) statute	Voucher specimen no.	Vernacular name (Melanesian language, or 'ncf', New Caledonia French)	Part(s) used and (use modality)	Traditional use in medicine (possibly TB, inflammation, infections) or other potential
		ndjii (ajië)	Leaves, mastication	Headache furonculosis, *rhumatisms*, as a diuretic against blennoragy
		ni (tîri)	Leaves, infusion	
		wênyi (ôrôê)	Leaves, scarification	
Zieridium melicopaefolium Guillaumin (Rutaceae) endemic	JWRG10	*whoo* (nemi)	Bark, decoction	*Cicatrisation* all disease gingivitis, *oedema* aperitive, digestif
		wayiü (cèmuhî)	Bbark, mastication	
		sôpiô (ajië)	Bark, infusion	

total extract was filtered and concentrated to dryness at reduced pressure.

2.2.2. Preparation with Soxhlet apparatus

Acronychia laevis (136 g, leaves), *Codiaeum peltatum* (61 g, stem bark), *Crossostylis multiflora* (102 g, leaves, 139 g stem bark) were successively extracted in Soxhlet apparatus with petroleum ether, dichloromethane and methanol. Filtered extracts were dried using a rotary evaporator under reduced pressure at 45 °C.

2.2.3. Preparation of essential oils

The essential oils of *Myoporum crassifolium* wood were obtained by hydrodistillation or by hexane Soxhlet extraction.

2.2.4. Determination of antimicrobial activity

The antimycobacterial activity of the extracts was determined against *Mycobacterium bovis* BCG (strain 11-73 P2, Institut Pasteur, Paris, France). *Mycobacterium bovis* was cultured in 7H9 Middlebrook medium (Difco) with 0.05% Tween 80 and 10% OADC (Oleic acid, Albumin Fraction V, Dextrose and Catalase) enrichment fluid (Difco) was added to the medium. Bacterial suspension test was adjusted to 10^5 cells/ml. Screening assays were performed in 96-well microplates. Fresh solutions of each extract were dissolved in DMSO. The plant extracts (1 mg/ml) were incorporated into the medium before solidification to obtain final concentration of 100, 50, 10, 5 2.5 and 1 μg/ml. Control experiments showed that a final concentration of DMSO (2%) did not affect the growth of *Mycobacterium bovis*. Twenty microliters with final concentration and 180 μl of mycobacteria in medium were added to each well and mixed to give a final volume of 200 μl/ml (10^5 CFU/ml *Mycobacterium bovis*). Reference drugs (pyrazynamide and ethambutol, Sigma–Aldrich) were included in a blank (culture medium only) and in a growth control medium and *Mycobacterium bovis* without the presence of drug). The plates were incubated for 5–6 days at 37 ± 1 °C. All tests were carried out in triplicate.

The minimum inhibitory concentration (MIC) of each extract corresponded to the lowest concentration at which the bacteria tested did not show growth.

3. Results and discussion

A total of 55 plant extracts from 20 different plant species (one with two varieties) belonging to 16 families were studied. Table 1 presents the botanical names and voucher specimens numbers, the vernacular names and their uses in traditional medicine, as known according to informations mainly collected by Dr. Dominique Cortadellas-Bourret (formerly at IRD Centre, Nouméa, New Caledonia) through interviews with local traditional healers. The results of the screening of crude plant extracts antimycobacterial activity are presented in Table 2. Various plants are used in the treatment of tuberculosis or its symptoms, among at which *Codiaeum peltatum*, *Drosera neocaledonica*. Five plant species (*Amborella trichopoda*, *Codiaeum peltatum*, *Myoporum crassifolium* and *Myristica fatua*, which belongs to the flora of Vanuatu, not New Caledonia) showed activity against *Mycobacterium bovis* BCG. Only the methanolic *Amborella trichopoda* extract (fruits) exhibited a pronounced antimycobacterial activity

Table 2
Screening of New Caledonian medicinal plant extracts for antimycobacterial activity

Plant name	Part used	Extract	Activity (MIC μg/ml)
Amborella trichopoda	Fruit	Methanol	1–2.5
	Stem	Dichloromethane	>100
		Methanol	50
	Leaf	Dichloromethane	>100
		Methanol	100
Codiaeum peltatum	Stem	Ether petroleum	>100
		Dichloromethane	>100
		Methanol	100
Myoporum crassifolium	Wood	Hexane	100
		Essential oils (hydrodistillation)	50
Myristica fatua	Almond	Dichloromethane	50
		Methanol	>100
	"Arille"	Dichloromethane	>100
		Methanol	>100
Reference drugs			
Ethambutol			20
Pyrazynamide			2.5

(MIC = 1–2.5 μg/ml) against mycobacteria. Five plant extracts presented a lower but interesting activity (MIC between 50 and 100 μg/ml), methanolic *Amborella trichopoda* extract (stem), ethanolic *s* (leaves) extract, methanolic *Codiaeum peltatum* extract (stem), two samples of *Myoporum crassifolium* essential oils (wood) and dichloromethane *Myristica fatua* extract (almond). However, it is interesting to note that the two samples of essential oils present a significant activity against mycobacteria, these extracts contain high concentration of terpenoids, which could explain their activity (Houghton et al., 1999). The different extracts prepared from plants used in traditional medicine to treat tuberculosis, such as *Drosera neocaledonica* and *Melodinus scandens* did not show any antimycobacterial activity at the test concentration. Although *Piper methysticum* root extracts is traditionally used for patients with pulmonary troubles their antimycobacterial activity was not substantiated.

In this study, *Amborella trichopoda* is the most active plant against mycobacteria. This plant is the only living member of Amborellaceae and recent phylogenetic analyses based on the comparison of a limited number of genes suggested that it is the most ancient angiosperm (Goremykin et al., 2003). In New Caledonia, information about its eventual uses in traditional medicine and its vernacular names was sought but has not yet been found.

Acknowledgments

Mycobacterium bovis strain was kindly provided by Dr. Gilles Marchal and Pierre Chavarot, Laboratory of Mycobacteria reference, Institute Pasteur, Paris, France.

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