World J Microbiol Biotechnol (2008) 24:569–572
DOI 10.1007/s11274-007-9494-4

ORIGINAL PAPER

Evaluation of antimicrobial activity of certain Chinese plants used in folkloric medicine

Airong Li · Yue Zhu · Xingbing He · Xingjun Tian · Liang Xu · Wu Ni · Ping Jiang

Received: 27 September 2006 / Accepted: 3 June 2007 / Published online: 26 July 2007

Abstract Six selected plants, belonging to 3 families from Nanjing of China, were extracted with the solvent 95% (v/v) ethanol to yield 11 extracts. The extracts were evaluated for their effects on the growth of eight clinical bacteria, two fungi and one yeast using a modified agar diffusion method. The results showed that the majority of the extracts investigated showed greater activities against the Gram-positive bacteria than against the Gram-negative bacteria, the fungi and the yeast. The strongest antimicrobial activity was exhibited by the stem extracts of *Mahonia fortunei* against multiresistant *Staphylococcus aureus* strains, followed by the stem extracts of *Mahonia bealei*, while *Bacillus thuringiensis* was the most sensitive to all extracts.

Keywords Antimicrobial activity · Chinese plants · Folkloric medicine · Microorganisms · Zone of inhibition

Introduction

In China, since antiquity, people have used plants to treat diseases, which contain what we would currently characterize as antimicrobial principles. The spread of traditional Chinese medicine to most continents has undoubtedly contributed to the current popularity of herbal medicines throughout the world (Gurib-Fakim 2006).

The plant samples studied in this paper were frequently used as materials to treat diseases. For example, camptothecin isolated from the extracts of *Camptotheca acuminata* was found to be an inhibitor of the growth of leukemia and cancer cells (Rivory and Robert 1995), nantenine from *Nandina domestica* to treat asthma, whooping cough, pharynx tumor and uterine bleeding in Japan (Tsuchida and Ohizumi 2003), *Mahonia* to contain an antitussive component, a glucuronoxylan (Kardošová et al. 2002), different parts of *Berberis* to be antibacterial, antipyretic, antipruritic and antiarrhythmic (Fatehi et al. 2005), *Lycoris* with a wide range of interesting physiological effects such as antitumor, antiviral (Yang et al. 2005). Within literatures related to species in this study, no one could be found in respect of antimicrobial activity of their ethanol extracts. This paper reports the first attempt to study the antimicrobial activity of ethanol extracts from different parts of six plants against microorganisms including eight clinical bacteria, two fungi and one yeast.

A. Li · Y. Zhu · X. He · X. Tian (✉) · L. Xu · W. Ni · P. Jiang
School of Life Science,
Nanjing University, Nanjing 210093, P.R. China
e-mail: tianxj@nju.edu.cn

Materials and methods

Plants collection and extraction procedure

The plant samples were *Lycoris chinensis* Traub., *Nandina domestica* Thunb., *Mahonia fortunei* (Lindl.) Fedde., *Mahonia bealei* (Fort.) Carr., *Berberis thunbergii* DC. cv. *atropurpurea* and *Camptotheca acuminata* Decne. Fresh plant materials were collected in November, 2005 from Nanjing, China. Voucher specimens were deposited in the Herbarium of the School of Life Science, Nanjing University, Nanjing, China.

Each 50 g air-dried and powdered material (with a 2 mm mesh) was extracted successively with 500 ml 95% (v/v) ethanol (CH_3CH_2OH) twice at room temperature. The macerated samples were filtered with Whatman filter paper

(no. 1). The supernatants were concentrated in vacuo at 60 °C using a rotary evaporator (RE-2000, Shanghai Yarong Instrument Factory, China) until a small volume (20–30 ml) was obtained. The concentrated extracts were dried in an oven at 60 °C to constant weight.

Microorganisms used and cultures

The bacteria used in this study including *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus thuringiensis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Enterobacter cloacae* were kindly provided by the Jiangsu Province Hospital of China. The fungi including *Aspergillus niger* and *Trichophyton rubrum* and the yeast Candida albicans were donated by Professor Liu, Microbiological Laboratory, Nanjing University, China.

The bacterial strains were cultured on nutrient agar medium. The fungi and the yeast were cultured on potato dextrose agar medium.

Antimicrobial assay

The dried extracts were dissolved in 10% (v/v) DMSO to a concentration of 300 mg/ml and sterilized by filtration with 0.45 μm Millipore filters. A modified agar diffusion method (NCCLS 1997) was used to determine the antimicrobial activity. Negative control was prepared using 10% DMSO. Gentamycin sulfate (Gen, Amerisco) (1 μg/disc) and nystatin (Nys, Amerisco) (20 μg/disc) were used as positive reference standards. The plates were inoculated at 37 °C for 24 h for bacterial strains, at 27 °C for 48 h for the yeast and 72 h for fungi. The diameters of the inhibition zones were measured by vernier caliper (Shangling 0–150 mm, Shanghai Everwin Tools Co., Ltd., China). Each test was performed in three replicates and repeated twice.

Results and discussion

The highest yield was obtained by the leaf extracts of *Nandina domestica* reached to 21.03% (w/w), followed by the fruits of *Camptotheca acuminata* (19.70%, w/w). The lowest was the stem extracts of *Nandina domestica* (4.68%, w/w), similar to the yield of *Mahonia fortunei* leaves (4.69%, w/w).

In this study, both antibacterial and antifungal activities were detected among crude extracts of *Lycoris chinensis* (bulbs), *Nandina domestica* (stems and leaves), *Mahonia fortunei* (stems and leaves), *Mahonia bealei* (stems and leaves), *Berberis thunbergii* (stems) and *Camptotheca acuminata* (stems, leaves and fruits) using a disc diffusion method on agar (Table 1).

Table 1 Antimicrobial activity of selected plants extracts based on a disc diffusion method (Zone of inhibition in mm)[a]

Plant species	Tested part	Yields (%)(w/w)	Tested microorganisms[c]										
			Gram(+)				Gram(–)				Fungi		Yeast
			B.s	S.a	S.f	B.t	E.c	P.a	S.d	E.cl	A.n	T.r	C.a
L .chinensis	Bulbs	7.71	–	–	–	9.21	–	–	–	–	–	–	–
N. domestica	Stems	4.68	–	10.09	8.83	10.84	–	–	–	–	–	–	–
	Leaves	21.03	–	–	–	7.20	–	–	–	–	–	–	–
M. fortunei	Stems	9.92	11.95	22.55	–	17.25	–	–	–	–	–	–	–
	Leaves	4.69	10.84	21.41	–	13.52	–	–	–	–	–	–	–
M. bealei	Stems	8.49	8.76	21.43	–	12.52	–	–	–	–	–	–	–
	Leaves	6.37	6.25	11.98	–	7.00	–	–	6.56	–	–	–	–
B. thunbergii cv.*atropurpurea*	Stems	5.37	6.77	19.78	–	16.34	–	–	–	–	–	–	–
C. acuminata	Stems	5.28	–	–	–	7.82	–	–	–	–	–	–	–
	Leaves	15.79	–	7.90	–	–	–	–	–	–	–	–	–
	Fruits	19.70	–	–	–	–	–	–	–	–	–	–	–
Standards[b]	Gen		23.32	–	–	17.24	17.53	–	14.76	–	NT	NT	NT
	Nys		NT	NT	NT	NT	NT	NT	NT	NT	12.81	10.84	10.01
	DMSO		–	–	–	–	–	–	–	–	–	–	–

[a] diameter of inhibition zones (mm) including disc diameter of 6 mm, "–": not active, NT: not tested

[b] Gen: gentamycin sulfate (1 μg/disc), Nys: nystatin (20 μg/disc)

[c] B.s: *Bacillus subtilis*, S.a: *Staphylococcus aureus*, S.f: *Streptococcus faecalis*, B.t: *Bacillus thuringiensis*, E.c: *Escherichia coli*, P.a: *Pseudomonas aeruginosa*, S.d: *Shigella dysenteriae*, E.cl: *Enterobacter cloacae*, A.n: *Aspergillus niger*, T.r: *Trichophyton rubrum*, C.a: *Candida albicans*

Most of plant extracts showed antibacterial activity against *Bacillus thuringiensis*, which was the most sensitive. *Mahonia fortunei* stem extracts displayed the widest zone of inhibition (22.55 mm in diameters). The most insensitive Gram-positive bacterium was *Streptococcus faecalis*, which was only sensitive to the extracts of *Nandina domestica* stems. The majority of the sample extracts did not display any antimicrobial activity against Gram-negative bacteria, except the leaf extracts of *Mahonia bealei* which inhibited weakly the growth of *Shigella dysenteriae*. It was interesting to note that the extracts of *Mahonia fortunei* (stems and leaves) and *Mahonia bealei* (stems) showed strong antimicrobial activity against the multiresistant *Staphylococcus aureus*, and the inhibition zones of them were more than 20 mm in diameters, followed by *Berberis thunbergii* stems extracts (19.78 mm in diameters). *Mahonia bealei* in southeastern United States (Allen et al. 2006) and *Berberis thunbergii* (Silander et al. 1999) in the New England were aggressive invaders. They may pose the greatest threat to biodiversity in local areas. On the basis of the present investigations, it can be highlighted that they can be cut and be exploited in herbal preparations at least for external uses to maintain the balance of ecology.

Although some species of *Lycoris* (Jia et al. 2001), *Nandina* (Indra et al. 2002), *Mahonia* (Kardošová et al. 2002), *Berberis* (Yeşilada and Küpeli 2002) and *Camptotheca* (Lorence and Nessler 2004) have been studied in a number of literatures and their medicinal values have been found, nothing is known about antimicrobial activities of these species reported here. Musumeci et al. (2003) reported that the root and leaf extracts of *Berberis aetnensis* C. Presl. generally showed a greater activity against Gram-positive than against Gram-negative, which was similar to the results of this paper. Freile et al. (2003) found that the aqueous extracts of *Berberis heterophylla* Juss. showed inactive against five microorganisms including *Staphylococcus aureus*, *Enterococcus faecali*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* in the concentration of 500 μg/ml and 1,000 μg/ml. In contrast with this paper, the extracts of *Berberis thunbergii* inhibited visibly the growth of *Staphylococcus aureus*. Furthermore, Li et al. (2006) reported that the minimum inhibitory concentration of stem extracts of *Berberis thunbergii* was 580 μg/ml.

The basis of sensitivity of test organisms may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds presented in the crude extracts. In present study, many alkaloids like galanthamine (Yang et al. 2005; Abdallah 1995) from *Lycoris*, nantenine (Tsuchida and Ohizumi 2003) from *Nandina domestica*, camptothecin (Pizzolato and Saltz 2003) from *Camptotheca acuminata*, and berberine from *Mahonia* (Ji et al. 2000) and *Berberis* (Fatehi et al. 2005) were detected in their extracts, and some of them have been proved efficacious (Zhu and Du 2006). The strong antimicrobial activities of *Mahonia fortunei*, *Mahonia bealei* and *Berberis thunbergii* may be mainly governed by their berberine. The active principles of these plant extracts and their safety as medicine may be tested further to uncover their therapeutic potential in modern and traditional medicine against infectious diseases.

Acknowledgments The authors would like to express their gratitude to Prof. Liu of Microbiological Laboratory, Nanjing University, China, and Jiangsu Province Hospital, Nanjing, China for their donation of microorganisms. The financial support of the National Natural Science Foundation of China (30470299) and Key Project of National Science Foundation (30430570) are also gratefully acknowledged.

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