Screening for Antimicrobial Activity of Some Medicinal Plants Species of Traditional Chinese Medicine

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Abstract

Janovská D., Kubíková K., Kokoška L. (2003): **Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine**. Czech J. Food Sci., **21**: 107–110.

The antimicrobial activity of crude ethanolic extracts of 10 medicinal plants used in traditional Chinese medicine was tested against five species of microorganisms: *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Of the 10 plants tested, 5 showed antimicrobial activity against one or more species of microorganisms. The most active antimicrobial plants were *Chelidonium majus*, *Sanguisorba officinalis*, and *Tussilago farfara*.

Keywords: antimicrobial activity; medicinal plants; crude extracts; traditional Chinese medicine

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al*. 2002).

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradski *et al*. 1999).

China throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases. Chemical studies of Chinese medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin (Qin & Xu 1998).

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al*. 1999).

In this study, ethanolic extracts of different parts (roots, rhizomes, aerial parts, leafs and fruits) of 10 plants, which had been described in herbal books and folklore medicine of China, were screened for their antimicrobial activity. The species tested were: *Achyranthes bidentata* Blume, *Belamcanda chinensis* (L.) DC., *Chelidonium majus* L., *Houttuynia cordata* Thunb., *Platycodon grandiflorum* (Jacq.)A.DC., *Rehmania glutinosa* (Gaertn) Steud., *Sanguisorba officinalis* L., *Schizandra chinensis* (Turcz.) Baill., *Tribulus terrestris* L., and *Tussilago farfara* L.

MATERIALS AND METHODS

Plant materials

The seeds of the plants tsted were obtained through Index Seminum from botanical gardens and universities (Jardin Botanique de la Ville et de l'Universite, France; Nasu Botanical Garden, Japan; The Nippon Shinyaku Institute for Botanical Research, Japan). They were grown in the experimental field of the Institute of Tropical and Subtropical Agriculture of the Czech University of

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. FRVŠ 1084.

Agriculture in Prague (ITSA CUA in Prague). The plant parts were collected during the months of May–September of 2001. Specimen samples were deposited at ITSA CUA in Prague.

Preparation of extract. Dried plant material (roots, rhizomes, aerial parts, leaves or fruits in lots 15 g) was macerated with 80% ethanol (450 ml) for five days and then filtrated. The filtrate was evaporated to a thick residue at 40°C. The residue was suspended or dissolved in 30 ml of Tris Buffer Saline (pH 7.6).

Micro-organisms tested. The following strains of bacteria were used: *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATTC 27853, and *Staphylococcus aureus* ATCC 25923 (Oxoid, England). The yeast strain used in this study was *Candida albicans* ATCC 10231 (Oxoid, England).

The micro-organisms were grown overnight at 37°C in Mueller-Hinton Broth (Oxoid, England) at pH 7.4. Their sensitivity to the reference antibiotics was checked (Table 2). Erythromycin and gentamycin (Sigma, USA) were used for the bacteria; amphotericin B (Sigma, USA) was used for the yeast.

Antibacterial testing. Antimicrobial activity of the crude ethanolic extracts of different plants was determined by the liquid dilution method (van den Berghe & Vlietinck 1991). Four-fold dilutions (three) of the tested extract sterilized by filtration through a 0.23 μm membrane filter were carried out starting from the dilution of 1/2. The tubes with the extract and broth were inoculated with a micro-organism suspension at a density of 10^5 CFU per ml. The tubes were incubated at 37°C for 24 h (or 48 h for the yeast) and then observed for the Minimum Inhibitory Concentration (MIC). The growth of organisms was observed as turbidity determined by a spectrophotometer (Ultrospec III, Pharmacia LKB, UK) at 620 nm. Control tubes without the tested extracts were assayed simultaneously. All samples were tested in triplicates.

RESULTS AND DISCUSSION

A total of 16 ethanolic extracts from 10 different plant species were investigated. The determination of the MIC by means of the liquid dilution method (Table 1) showed that 5 plant extracts tested exhibited an antimicrobial effect against some of the five tested micro-organisms.

The results showed that the extracts from *Sanguisorba officinalis*, *Tussilago farfara* (aerial part; rhizome), *Chelidonium majus* (root), *Tribulus terrestris* (aerial part) and *Schisandra chinensis* (leaves) possessed antimicrobial activity.

Although the plants differ significantly in their activities against the micro-organisms tested, more of the extracts showed antimicrobial activity against *B. cereus* and *S. aureus* than against *E. coli*, *P. aeruginosa* and *C. albicans*.

According to the liquid dilution screening method for antimicrobial activity of higher plants reported by van den Berghe and Vlietinck (1991), a prominent antibacterial effect, worthy of further investigation, is obtained if not only the 1/2, but also the 1/8 and 1/32 dilutions show inhibitory activities. An inhibition shown for the 1/2 dilution only is less promising for further investigation.

From this study we can conclude that the plants *C. majus*, *S. officinalis*, and *T. farfara* possessed the highest antimicrobial activity. All these species are perennial herbs widely used as medicines and described in the Chinese Materia Medica (Benski & Gamble 1993).

Antiviral activities of extracts isolated from *S. officinalis* were previously reported (Kim *et al*. 2001). Extracts from fruits of *S. chinensis* separated into *n*-butanol and diethyl ether showed antagonistic effects on *Alternaria alata* (Kim *et al*. 1996).

The methanolic extract of the roots of *Ch. majus* revealed a high resistance to *Fusarium* (Matos *et al*. 1999). Several flavonoids and phenolic acids were isolated from the aerial parts which exhibit interesting antiviral and antimicrobial properties both *in vitro* and *in vivo* (Colombo & Bosisio 1996). A glycoprotein isolated from *C. majus* exhibits good antibacterial activity against methicillin resistant staphylococci and multiresistant enterococci (Fik *et al*. 1997).

Medicinal plant can be poisonous if wrong plant parts or wrong concentrations are used (Frohne 1999). Some compounds from plants may be toxic in higher doses. Tussilagone isolated from *T. farfara* is a potent cardiovascular and respiratory stimulant but it has LD_{50} in mice 28.9 mg/kg (Li & Wang 1988). Plants containing pyrrolizidine alkaloids (*T. farfara*) could be toxic for man or livestock (Koch *et al*. 1994). The hepatotoxic potential of conventional drugs is well known while herbal medicines are often assumed to be harmless. *C. majus* is frequently prescribed to treat gastric and biliary disorders but it may be the cause of cholestatic hepatitis (Benninger *et al*. 1999).

Table 1. Antibacterial activity of ethanol crude extracts of some medicinal plants of traditional Chinese medicine

Species (family) and voucher specimen number	Folk medicine use	Part tested	Micro-organisms[a]/MIC[b]				
			Bc	*Ca*	*Ec*	*Pa*	*Sa*
Achyranthes bidentata Blume (*Amaranthaceae*) 0109	diuretic; emmenagogue	aerial part	n.a.	n.a.	n.a.	n.a.	n.a.
Belamcanda chinensis (L.)DC. (*Iridaceae*) 0192	antipyretic; expectorant	aerial part	n.a.	n.a.	n.a.	n.a.	n.a.
Chelidonium majus L. (*Papaveraceae*) 0134	cholagogue; spasmolytic; analgetic	aerial part	n.a.	n.a.	n.a.	n.a.	n.a.
		root	15.63	62.50	n.a.	n.a.	62.50
Houttuynia cordata Thunb. (*Saururaceae*) 0143	diuretic; antiseptic	aerial part	n.a.	n.a.	n.a.	n.a.	n.a.
		rhizome	n.a.	n.a.	n.a.	n.a.	n.a.
Platycodon grandiflorum (Jacq.) A. DC. (*Campanulaceae*) 0119	expectorant	aerial part	n.a.	n.a.	n.a.	n.a.	n.a.
		root	n.a.	n.a.	n.a.	n.a.	n.a.
Rehmannia glutinosa (Gaertn) Steud. (*Scrophulariaceae*) 0151	cardiotonic; diuretic	root	n.a.	n.a.	n.a.	n.a.	n.a.
Sanguisorba officinalis L. (*Rosaceae*) 0125	anti-haemorrhage; antiphlogistic	aerial part	62.50	n.a.	250.00	250.00	62.50
		rhizome	15.63	250.00	250.00	62.50	250.00
Schizandra chinensis (Turcz.) Baill. (*Magnoliaceae*)	tonic; stimulant; antitussive	leaf	250.00	n.a.	n.a.	n.a.	n.a.
Tribulus terrestris L. (*Zygophyllaceae*) 0156	astringent; tonic	aerial part	n.a.	n.a.	n.a.	n.a.	250.00
		fruit	n.a.	n.a.	n.a.	n.a.	n.a.
Tussilago farfara L. (*Compositae*) 0117	antiseptic; antiphlogistic	aerial part	15.63	n.a.	n.a.	n.a.	62.50
		rhizome	62.50	n.a.	n.a.	n.a.	62.50

[a]Micro-organisms: *Bc* – *Bacillus cereus*; *Ca* – *Candida albicans*; *Ec* – *Escherichia coli*; *Sa* – *Staphylococcus aureus*; *Pa* – *Pseudomonas aeruginosa*

[b]MIC – Minimum Inhibitory Concentration (mg of dry plant material/ml); n.a. – not active

Table 2. Antimicrobial reference standards

Antibiotic	Micro-organism[a]/MIC[b]				
	Bc	*Ca*	*Ec*	*Pa*	*Sa*
Amphotericin B	–	0.39	–	–	–
Erythromycin	0.78	–	–	–	1.56
Gentamicin	–	–	1.56	3.13	–

[a]Micro-organisms: see Table 1; [b]MIC – Minimum Inhibitory Concentration (μg/ml); – not determined

Despite many published reports dealing with bioactivity of compounds isolated from *T. farfara*, little was known about its antimicrobial activity prior to our investigation.

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Received for publication October 28, 2002
Accepted after corrections May 28, 2003

Souhrn

Janovská D., Kubíková K., Kokoška L. (2003): **Stanovení antimikrobiální aktivity u vybraných druhů léčivých rostlin tradiční čínské medicíny**. Czech J. Food Sci., **21**: 107–110.

Cílem studie bylo stanovení antimikrobiální aktivity 16 hrubých extraktů z 10 druhů rostlin užívaných v tradiční čínské medicíně. Testována byla aktivita proti pěti druhům mikroorganismů (*Bacillus cereus*, *Escherichia coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa* a *Candida albicans*). Z deseti testovaných rostlin pět vykázalo významnou antimikrobiální aktivitu. Nejvýraznější účinky měly *Chelidonium majus*, *Sanguisorba officinalis* a *Tussilago farfara*.

Klíčová slova: antimikrobiální aktivita; léčivé rostliny; hrubý extrakt; tradiční čínská medicína

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