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

Dagmar JANOVSKÁ, Kateřina KUBÍKOVÁ and Ladislav KOKOŠKA

Department of Tropical and Subtropical Crops, Institute of Tropical and Subtropical Agriculture, Czech University of Agriculture, Prague-Suchdol, Czech Republic

Abstract


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 (Steradski 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, leaves 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 tested were obtained through Index Seminum from botanical gardens and universities (Jardin Botanique de la Ville et de l’Université, 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 Agriculture.
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 ATCC 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 Bergh & 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 Bergh 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 (Mapos 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 & Bosiso 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

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

\(^a\)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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Micro-organism(^a)/MIC(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bc</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.78</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\)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.

References

Benninger J., Schneider H.T., Schuffan D., Kirchner T., Hahn E.G. (1999): Acute hepatitis induced by...
Souhrn


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

Corresponding author:
Ing. Ladislav Kokoška, Ph.D., Česká zemědělská univerzita v Praze, Institut tropického a subtropického zemědělství, Kamýcká 129, Praha 6-Suchdol, Česká republika
tel.: +420 224 382 180; fax: +420 220 921 363, e-mail: kokoska@itsz.czu.cz