ORIGINAL SCIENTIFIC PAPER

The possibility of using plant extracts in control of Agrobacterium tumefaciens (Schmit and Townsend) Conn

Aleksandra Stanojković¹, Radmila Pivić¹, Dragana Jošić¹, Aleksandar Stanojković²

¹ Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia (aleksandra-s@sbb.rs)
² Faculty of Veterinary Medicine, University of Belgrade, Serbia

Abstract

For the purpose of finding the possibility of using plant extracts in biocontrol of Agrobacterium tumefaciens, in vitro antibacterial activity of the water, ethanol and ethyl-acetate extracts of selected plants from the family Asteraceae was investigated. The effect of extracts on bacterium was determined by filter disc diffusion method. The extracts of A. millefolium, H. arenarium, A. absinthium, C. cyanus, M. chamomilla and T. officinale significantly inhibited the tested bacterium. The obtained results indicate the possibility of formulation and application of plant preparations made from extracts of these plants in biological control of A. tumefaciens.

Key words: Agrobacterium tumefaciens, plant extracts, antibacterial activity

Introduction

Bacterium Agrobacterium tumefaciens (Schmit and Townsend) Conn invades the crown, roots and stems of a great variety of dicotyledonous and some gymnospermous plant causing disease called crown gall, hairy root or cane gall (Holt et al., 1994). It overwinters in infested soils, where it can live as a saprophyte for several years. Once inside the plant tissue, the bacterium moves from cell to cell, stimulating surrounding host cells to divide at a rapid rate. Young, soft galls are easily injured and attacked by insects and saprophytic microbes, which cause the outside cell layers to decay and discolor. The breakdown of the gall releases bacteria back into the soil, and the bacteria are free to infect new plants (Alsup, 2004). There are no chemical pesticides to control A. tumefaciens, but biological control does provide some protection. Before planting, roots and crown can be dipped in a solution containing genetically modified related bacteria, Agrobacterium radiobacter, strain 84, that protect against infection (Lopez et al., 1989).

Although approximately 20% of the world plants have been submitted to pharmacological or biological test, it could be concluded that natural products from plant origin are an important source to discover new leads with economical and pharmaceutical importance and great possibilities to be developed as drugs, dyes, fragrances and pesticides (Hamburger and Hostettman, 1991). For the purpose of finding the possibility of using plant extracts in biocontrol of A. tumefaciens, antibacterial activity of the water, ethanol and ethyl-acetate extracts of selected aromatic and medicinal plants from the family Asteraceae was investigated in this study under in vitro conditions.

Material and methods

The plant species studied for their antibacterial activity in this investigation are listed in Table 1. The plant material was collected in the period of January – October 2002, on three different locations of the republic of Serbia (the mountains Zlatibor and Tara, and the
environment of the town of Zaječar) and identified at Faculty of Science, University of Kragujevac (Serbia). Eleven plant species were collected in the phase of blooming, while *T. officinale* was collected in the phase before the flowery heads had been formed. The water, ethanol and ethyl-acetate extracts were made by boiling dry, macerated plant (30g for each solvent) at the water bath at the temperature of 80 °C for 1 hour. The filtration and evaporation were done after 24 hours. The ethanol and ethyl-acetate solutions were evaporated in vacuum at 40 °C, while the evaporation of the water solution was done at the water bath. The solutions were evaporated until the dry extracts were obtained. The suitable solvents (water, ethanol, ethyl-acetate) in the amount of 50 ml were added into the obtained extracts. Related to the mass of dry matter (mg), the concentrations of the extracts for antibacterial investigation (ml) were calculated.

Effect of the plant extracts on *A. tumefaciens* was determined by filter disc diffusion method (Uhlik, 1972). The inoculation was carried out by transferring 0.1 ml of bacterial suspension into Petri dishes with 10 ml of the nutrient media. The sterile filter-paper discs (ø 10 mm) saturated with extracts in the concentration of 5, 10 and 15 mg/disc were placed over the surface of the inoculated nutrient media. The incubation was carried out in the thermostate at 24°C. The measuring of the inhibition zones was done after 24 hours and carried out in six repetitions, on the basis of which the average values were obtained.

**Results and discussion**

Inhibitory effect of the tested extracts on *A. tumefaciens* is presented in Table 1 and Graphs 1, 2 and 3, as well as figuratively (Picture 1). By processing the obtained data it was determined that all the tested extracts of *A. millefolium* and *H. arenarium*, then, water and ethanol extracts of *C. cyanus* and *M. chamomilla*, as well as *A. absinthium* and *T. officinale* water extracts significantly inhibited the tested bacterium. In general, water and ethanol extracts were more active than ethyl-acetate extract. The maximal tested concentrations (15 mg/disc) showed the highest antibacterial activity. *H. arenarium* ethanol extract in concentration of 5 mg/disc inhibited this bacterium in zone of 5 mm, and in concentration of 15 mg/disc in zone of 8.08 mm. *A. millefolium* ethyl-acetate extract inhibited the tested bacterium in zones of 0.58-5.16 mm.

Extracts and secondary metabolites of many plant species were reported to have high biocontrol efficacy under laboratory or in greenhouse conditions (Klingauf, 2005). The phytochemical screening on the Asteracea family has revealed sesquiterpene lactones as the principal secondary metabolites responsible for their antimicrobial activities. Plants of the genus *Artemisia* produce many eudesmanolides, the group of sesquiterpene lactones that exhibit significant antibacterial properties (Teixeira da Silva, 2004). *Artemisia absinthium* essential oil was active against bacterial strains that cause bacterial diseases in plants (Kokošková and Pavela, 2005). The capacity of essential oils of the *Achillea* species to inhibit *A. tumefaciens* was previously reported and provides a natural alternative to conventional *Agrobacterium*-eliminating antibiotics (Al Kurdi et al., 2000). Some members of the genus *Helichrysum* have been well characterized with respect to their secondary metabolites, largely dominated by alkaloids, flavonoids, phloroglucinols and tannins with antibacterial properties (Jakupović et al., 1989; Mathekga and Meyer, 1998). Interest in the genus *Matricaria* has increased since its constituents, among others, have high antimycobacterial and antimicrobial agents (Aggag and Yousef, 1972).

As an alternative strategy to prevent the spread of diseases on cultured plants, natural compounds of plants can be a source of new biopesticides. The obtained results in this study present the preliminary observation in order to select among crude plant extracts those with potentially useful properties for further chemical investigation. It indicates the
possibility of formulation and application of plant preparations in biological control of *A. tumefaciens*.

Table 1. Average values of inhibition zones of *A. tumefaciens* growth expressed in mm

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Agrobacterium tumefaciens</th>
<th>Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Ethanol</td>
</tr>
<tr>
<td><strong>Plant species</strong></td>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>2.50&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.00</td>
</tr>
<tr>
<td><em>Artemisia absinthium</em> L.</td>
<td>0.77&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.63</td>
</tr>
<tr>
<td><em>Calendula officinalis</em> L.</td>
<td>1.67</td>
<td>3.33</td>
</tr>
<tr>
<td><em>Centaurea cyanus</em> L.</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td><em>Cichorium intybus</em> L. (aerial parts)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Cichorium intybus</em> L. (root)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Eupatorium cannabinum</em> L.</td>
<td>1.83</td>
<td>4.58</td>
</tr>
<tr>
<td><em>Helichrysum arenarium</em> (L.) DC</td>
<td>0.68</td>
<td>0.49</td>
</tr>
<tr>
<td><em>Inula helenium</em> L.</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em> L.</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Solidago virga-aurea</em> L.</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em> Weber</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Tussilago farfara</em> L.</td>
<td>1.00</td>
<td>2.67</td>
</tr>
</tbody>
</table>

<sup>3</sup>Concentrations: C<sub>1</sub>=5mg/disc, C<sub>2</sub>=10mg/disc, C<sub>3</sub>=15mg/disc; <sup>3</sup>STDEV

Graph 1. Antibacterial activity of the plant extracts in the concentration of 5 mg/disc
Graph 2. Antibacterial activity of the plant extracts in the concentration of 10 mg/disc

Graph 3. Antibacterial activity of the plant extracts in the concentration of 15 mg/disc

Picture 1. Inhibition zones of *A. tumefaciens* growth caused by *H. arenarium* ethanol extract (conc. 15 mg/disc)

**Conclusions**

On the basis of the obtained results it can be concluded that all the tested extracts of *A. millefolium* and *H. arenarrium*, then, water and ethanol extracts of *C. cyanus* and *M. chamomilla*, as well as *A. absinthium* and *T. officinale* water extracts significantly inhibited *A. tumefaciens*. The maximal tested concentrations (15 mg/disc) showed the strongest antibacterial effect. This study indicates the possibility of formulation of plant preparations and their application in biological control of *A. tumefaciens*. 
References


