ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS AGAINST DIFFERENT STRAINS OF BACTERIA

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ABSTRACT

In this study, methanolic extracts of Tilia cordata Mill. and Aesculus hippocastanum which had been described in herbal books, were screened for their antimicrobial activity against gramnegative and grampositive bacteria. The following strains of bacteria for antimicrobial activity were used gramnegative bacteria: Escherichia coli CCM 3988, Listeria ivanovii CCM 5884, Listeria innocua CCM 4030, Pseudomonas aeruginosa CCM 1960, Serratia rubidaea CCM 4684 and grampositive bacteria: Brochothrix thermosphacta CCM 4769, Enterococcus raffinosus CCM 4216, Lactobacillus rhamnosus CCM 1828, Paenobacillus larvae CCM 4483 and Staphylococcus epidermis CCM 4418 using disc diffusion method and microbroth dilution technique according to CLSI. Probit analysis was used in this experiment. Of the 2 plant extracts tested, all showed antimicrobial activity against one or more species of microorganisms. The highest antibacterial activity of Tilia cordata and Aesculus hippocastanum methanolic extract was measured against gramnegative bacteria Pseudomonas aeruginosa used with disc diffusion method. The strong antimicrobial activity with microbroth dilution method of Tilia cordata and Aesculus hippocastanum were found against Listeria ivanovii.

Keywords: Tilia cordata, Aesculus hippocastanum, methanolic extracts, gramnegative and grampositive bacteria

INTRODUCTION

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases (Mathana et al., 2010). Approximately 60%-80% of the world’s population still relies on traditional medicine for the treatment of common illnesses (WHO, 2007; Dev, 2010; Schuster and Wolber, 2010). And about 60%-90% of patients with arthritis who have used complementary and alternative medicine, most used Traditional Chinese medicine (Tsang, 2007).

Aesculus hippocastanum (family Hippocastanaceae) is commonly known as Horse chestnut, which is native to Western Asia. The extracts of Horse chestnut have been traditionally employed both in the West and East for the treatment of peripheral vascular disorders including haemorrhoids, varicose veins, leg ulcers and bruises (Evans, 2002). It is used in the treatment for chronic venous insufficiency and peripheral edema (Sirtori, 2001). It is also used for the prevention of gastric ulcers, reduction of cerebral edema, reduction of cellulite, as adrenal stimulant, hypoglycemic agent, antithrombotic, antiinflammatory, and also for reduction of hematomas and inflammation from trauma or surgery. Active Chemical Constituents of horse chestnut are coumarin derivatives like aucuslín, fraxín, scopólin; flavonoids like quercetin, kaempferol, etin, isoquercetin, rutin, leucocyanidin and essential oils like oleic acid, linoleic acid. Other constituents include amino acids (adenosine, adonine, guanine), allantoín, argyrín, carotín, choline, citric acid, epicatechín, leucodelphinidnín, phystöler, resin, scopólatin, tannin, and uric acid (Roy et al., 2011). The principal extract and medicinal constituent of horse chestnut seed is escin, a mixture of triterpenoid saponin glycosides. Its components include protoaescigenin, bartzingotenol C, allantoín, sterols, leucocyanidin, leucodelphiñin, tannins, and alkanes (Roy et al., 2011). In common with the bark of A. hippocastanum, leaf tissues contain the coumarin glycosides scopólin, fraxín and esculín. A range of flavonoid glycosides of quercetin (e.g. quercetín, rutin, isoquercetín and quercetín 3-arabinosíd) and the corresponding glycosides of kaemperfol have also been detected in leaf tissues. In addition to these glycosides, escin has been detected (but only in trace amounts), as well as leucanthochyans, cis,trans-polyprenols, amino acids, fatty acids and sterols (sitosterol, stigmatosterol and campesterol) (Kukric et al., 2013).

Tilia cordata Mill. (Tiliaceae) has been used in folk medicine, primarily as a non-narcotic sedative for sleep disorders or anxiety. The anxiolytic effect of Tilia species, such as T. americana var. Mexicanus, has been attributed to the presence of tiloside (Perez-Ortega et al., 2008). Phytochemical studies have demonstrated that Tilia species possess hydrocarbons, esters, aliphatic acids (Fitsiou et al., 2007), terpenoids, quercetin and kaempferol derivatives, phenolic compounds, condensed tannins (Behrens et al., 2003) and a coumarin scopoletin (Arcos et al., 2006). Tilia americana var. Mexicanus has several flavonoids such as rutin, hyperoside, quercetin and tiloside (Aguirre-Hernandez et al., 2010). Consumers life is about changes and development. In some cases, it is question of comeback, in another ones the question of futuristic wishes. Nevertheless, the only important thing is to satisfy our customer, but nowadays, do not forget sustainability issues in broaden understanding (Horská, 2012).

The present study was designed to determine the role of methanolic extracts of Tilia cordata and Aesculus hippocastanum for potential antibacterial activity against some selected microorganisms as gramnegative bacteria: Escherichia coli CCM 3988, Listeria ivanovii CCM 5884, Listeria innocua CCM 4030, Pseudomonas aeruginosa CCM 1960, Serratia rubidaea CCM 4684 and grampositive bacteria: Brochothrix thermosphacta CCM 4769, Enterococcus raffinosus CCM 4216, Lactobacillus rhamnosus CCM 1828, Paenobacillus larvae CCM 4483 and Staphylococcus epidermidis CCM 4418.

MATERIAL AND METHODS

Preparation of crude extracts

Leaves samples of Tilia cordata Mill. and Aesculus hippocastanum were dried and the dried material was ground to a coarse powder. Fifty grams of the sample of dried plant material was extracted extensively in 150 ml ethanol for two weeks at room temperature with gentle shaking. The extract was filtered through filter...
paper (Whatman no. 54) under vacuum followed by drying by rotary evaporation. Detailed information about medical plants shows tab. 1.

### Tested microorganisms

The following strains of bacteria were used Gramnegative bacteria: Escherichia coli CCM 3988, Listeria ivanovii CCM 5884, Listeria innocua CCM 4030, Pseudomonas aeruginosa CCM 1960, Serratia rubidaea CCM 4684 and grampositive bacteria: Brochothrix thermosphacta CCM 4769, Enterococcus raffinosus CCM 4216, Lactobacillus rhamnosus CCM 1828, Paenibacillus larvae CCM 4483 and Staphylococcus epidermis CCM 4418. The bacterial strains were purchased from the Czech Collection of Microorganisms (CCM). The microorganisms were grown overnight at 37 °C in Mueller-Hinton Broth (Oxoid, England) at pH 7.4.

### Antibacterial activity with disc diffusion method

Antimicrobial activity of each plant extract was determined using a disc diffusion method. Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells ml^-1. One hundred microlitres of the microbial suspension were spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

### Minimum inhibitory concentration MIC

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique, using 96-well microtiter plates. The bacterial inoculum applied contained approximately 1.0 x 10^6 cells in a final volume of 100 µl.well^-1. The pure plant material tested were dissolved in DMSO (512 to 1 µg.ml^-1) and added to broth medium with bacterial inocula. The microplates were incubated for 16 – 20 hours at 37 °C. The lowest concentrations without visible growth determined as different between start concentration and final concentration of solution by ELISA Reader (Biotek ELx808U) were defined as concentrations which completely inhibited bacterial growth (MICs). The first row on 96-well microtiter plate was control of sterility and final row was control of growth without pure compound of plant material.

### Statistical analysis

From obtained measured absorbances before and after this experiment we changed differences in absorbance between measuring to set of binary values. These values were assigned exact concentrations. For this experiment we created follow formula: if absorbance values were a lower as 0.05 then numbers for binary system were 1 (inhibitory effect), if absorbance values were a higher as 0.05 than numbers for binary system were 0 (no effect or stimulant effect). For this statistical evaluation Probit analysis in Statgraphic software was used.

### RESULTS AND DISCUSSION

In Europe, the bark, leaves, horse chestnut seed extract (HCSE), and aescin (a saponin mixture) from A. hippocastanum have been used in the treatment of chronic venous insufficiency, hemorrhoids, and postoperative edema (Khan, 2006; Persson and Persson, 2010). In China, the seeds of A. chinensis var. chinensis have been used as a stomachic and analgesic in the treatment of diarrhoea and pain in chest and abdomen, malaria, and dysentery and tablets made from the seeds are also used for treating heart diseases. Modern pharmacologic investigations have confirmed that HCSE, aescin and individual compounds isolated and identified from the two Eurasian species and other Aesculus species possess diverse activities, including anti-inflammatory, antitumor, antiviral, antioxidant, and antigenotoxic properties. The chemical constituents of some Aesculus species have been well documented. To date, more than 210 compounds from different classes have been isolated and identified from the genus Aesculus. These compounds include triterpenoids, triterpenoid glycosides (saponins), flavonoids, coumarins, carotenoids, long fatty chain compounds, and some other classes of compounds (Zhang et al., 2010).

The in vitro antibacterial activity of the Tilia cordata and Aesculus hippocastanum methanolic extracts was tested by using disc diffusion method with the microorganisms as seen in table 2. The highest antibacterial activity of Tilia cordata methanolic extract was measured against grammegative bacteria Pseudomonas aeruginosa (8 mm) and highest antibacterial activity of Aesculus hippocastanum methanolic extracts was measured against Pseudomonas aeruginosa (2.3 mm) too used with disc diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Medicinal plant extract</th>
<th>Mean (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli  CCM 3988</td>
<td>Tilia cordata</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>P. aeruginosa CCM 1960</td>
<td>Tilia cordata</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>2.30</td>
</tr>
<tr>
<td>S. rubidaea CCM 4684</td>
<td>Tilia cordata</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>Listeria ivanovii CCM 5884</td>
<td>Tilia cordata</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>Listeria innocua CCM 4030</td>
<td>Tilia cordata</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>E. raffinosus CCM 4216</td>
<td>Tilia cordata</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>B. thermospacta CCM 4769</td>
<td>Tilia cordata</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
<tr>
<td>S. epidermis CCM 4418</td>
<td>Tilia cordata</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>2.00</td>
</tr>
<tr>
<td>L. rhamnosus CCM 1828</td>
<td>Tilia cordata</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>2.30</td>
</tr>
<tr>
<td>P. larvae CCM 4483</td>
<td>Tilia cordata</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>Aesculus hippocastanum</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The determination of the MIC by means of the microbroth dilution method (tab. 3) showed that plant extracts tested exhibited an antimicrobial effect against some of the ten tested microorganisms. The strong antimicrobial activity of Tilia cordata and Aesculus hippocastanum were found against Listeria ivanovii.
Horse chestnut seed extract is found to be active against oral microbes like Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis, Streptococcus sanguis and Lactobacillus acidophilus (Roy et al., 2011). In the study of Özbucak et al. (2013), the antimicrobial capacity of the extracts from the flower and leaf of T. rubra subsp. caucasica against bacteria and fungi were determined. The antimicrobial activity of the extracts of flower and leaf was more effective against bacteria than fungi, similar to the results of Avato et al., (1997) and Zavala and Perez (1997). But the antimicrobial activity of the extracts of bark from Tilia species was more effective against fungi than bacteria (Toker et al., 1995).

CONCLUSION

This in vitro study demonstrated that folk medicine can be effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established. It is clear from the results that, the extracts act as a good source of an antimicrobial agent against Pseudomonas aeruginosa and Listeria ivanovii.

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