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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 extracts 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*, metnanolic extracts, gramnegative and grampositive bacteria

INTRODUCTION

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases (**Mothana *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 *Hippocastanacae*) 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 aesculin, fraxin, scopolin; flavonoids like quercetin, kaempferol, astragalin, isoquercetrin, rutin, leucocyanidine and essential oils like oleic acid, linoleic acid. Other constituents include amino acids (adenosine, adenine, guanine), allantoin, argyrin, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin, and uric acid (**Roy *et al.*, 2011**). The principal extract and medicinal constituent of horse chestnut seed is aescin, a mixture of triterpenoid saponin glycosides. Its components include protoaescigenin, barringtogenol C, allantoin, sterols, leucocyanidin, leucodelphinidin, tannins, and alkanes (**Roy *et al.*, 2011**). In common with the bark of *A. hippocastanum*, leaf tissues contain the coumarin glycosides scopolin, fraxin and esculin. A range of flavonoid glycosides of quercetin (e.g. quercitrin, rutin, isoquercitrin and quercetin 3-arabinoside) 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 leucanthocyans, cis,trans-polyprenols, amino acids, fatty acids and sterols (sitosterol, stigmasterol 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. *Mexicana*, has been attributed to the presence of tiliroside (**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. *Mexicana* has several flavonoids such as rutin, hyperoside, quercitrin and tiliroside (**Aguirre-Hernandez *et al.*, 2010**).
Consumers life is about changes and development. In some causes, 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 epidermis* 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.

Table 1 Detail information about plants and plant extracts

Orig. Latin title	Plant parts	Yield	Area	Dissolving time	Extracted by
Tilia cordata	flower	1815.1	Nitra	2 weeks at room temperature	Vacuum evaporator from methanol at room temperature at -800 mbar
Aesculus hippocastanum	flower	509.5	Nitra		

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, *Paenobacillus 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 10^5 cells.ml^{-1}. One hundred microlitres of the microbial suspension was 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 microtitrate plates. The bacterial inoculum applied contained approximately 1.0 x 10^5 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 ELx808iU) were defined as concentrations which completely inhibited bacterial growth (MICs). The first row on 96-well microtitrate 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 followed formula: if absorbance values were a lower as 0.05 than 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 distention 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, antioxidative, 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 were 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 gramnegative 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 2 Antibacterial activity of medicinal plants against bacteria in mm

Microorganism	Medicinal plant extract	Mean (mm)
***E. coli* CCM 3988**	**control**	0.00
	Tilia cordata	2.00
	Aesculus hippocastanum	0.00
***P. aeruginosa* CCM 1960**	**control**	0.00
	Tilia cordata	8.00
	Aesculus hippocastanum	2.30
***Seratia rubidae* CCM 4684**	**control**	0.00
	Tilia cordata	0.00
	Aesculus hippocastanum	0.00
***Listeria ivanovii* CCM 5884**	**control**	0.00
	Tilia cordata	0.00
	Aesculus hippocastanum	0.00
***Listeria innocua* CCM 4030**	**control**	0.00
	Tilia cordata	0.00
	Aesculus hippocastanum	0.00
***E. raffinosus* CCM 4216**	**control**	0.00
	Tilia cordata	0.00
	Aesculus hippocastanum	0.00
***B. thermospacta* CCM 4769**	**control**	0.00
	Tilia cordata	2.00
	Aesculus hippocastanum	0.00
***S. epidermis* CCM 4418**	**control**	0.00
	Tilia cordata	4.30
	Aesculus hippocastanum	2.00
***L. rhamnosus* CCM 1828**	**control**	0.00
	Tilia cordata	0.00
	Aesculus hippocastanum	2.30
***P. larvae* CCM 4483**	**control**	0.00
	Tilia cordata	2.30
	Aesculus hippocastanum	0.00

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*.

Table 2 Determined MICs value for selected medical plants (MeOH extracts) to gramnegative and gram positive microorganisms

Abr.*	Microorganisms	Antimicrobial activity of medicinal plants extract (µg.mL^{-1})			
		Tilia cordata		*Aesculus hippocastanum*	
		MIC 50	MIC 90	MIC 50	MIC 90
Gramnegative microorganisms					
Liv	*Listeria ivanovii* CCM 5884	96.04	102.53	383.65	407.95
Sr	*Serratia rubidaea* CCM 4684	383.65	407.95	634.57	1170.20
Lin	*Listeria innocua* CCM 4030	766.01	814.26	> 1024	> 1024
Ec	*Escherichia coli* CCM 3988	> 1024	> 1024	> 1024	> 1024
Pa	*Pseudomonas aeruginosa* CCM 1960	> 1024	> 1024	> 1024	> 1024
Grampositive microorganisms					
Er	*Enterococcus raffinosus* CCM 4216	> 1024	> 1024	> 1024	> 1024
Lr	*Lactobacillus rhamnosus* CCM 1828	> 1024	> 1024	> 1024	> 1024
Se	*Staphylococcus epidermis* CCM 4418	> 1024	> 1024	> 1024	> 1024
Bt	*Brochothrix thermosphacta* CCM 4769	> 1024	> 1024	> 1024	> 1024
Pl	*Paenobacillus larvae* CCM 4483	> 1024	> 1024	> 1024	> 1024

*Abreviations, (MICs determined by Probit analysis, $p< 0.05$)

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 as 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 antimicrobial agent against *Pseudomonas aeruginosa* and *Listeria ivanovii*.

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