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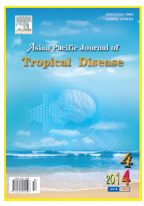

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Phenolic profile and antimicrobial activities to selected microorganisms of some wild medical plant from Slovakia

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PEER REVIEW

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Comments

The described research fits the global trend of looking for natural medicines with antibiotic properties. Tested plants are known in folk and traditional medicine but not as antibacterial agents. Study has been carried out properly and the description of the results is concise and clear.
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ABSTRACT

Objective: To investigate the chemical composition and antimicrobial activity of the methanol extracts of *Tussilago farfara (T. farfara)*, *Equisetum arvense*, *Sambucus nigra (S. nigra)* and *Aesculus hippocastanum*.

Methods: The antimicrobial activities of the extracts against *Enterococcus raffinosus*, *Escherichia coli*, *Lactobacillus rhamnosus*, *Pseudomonas aeruginosa*, *Serratia rubidaea*, *Saccharomyces cerevisiae* and *Staphylococcus epidermis* were determined by the microbroth dilution method according to Clinical and Laboratory Standards Institute, while the concentrations of main phenolic acids and flavonoids in the form of trimethylsilyl ethers were analysed using gas chromatography-mass spectrometry. The probit analysis was used for statistical evaluation.

Results: Of the 4 plant tested, all extracts showed a significant antimicrobial activity against one or more species of examined microorganisms. The most active antimicrobial plant extract was gathered from *T. farfara*, followed by *Aesculus hippocastanum* and *Equisetum arvense*. The extract from *S. nigra* showed no antimicrobial effects. The flavonoids quercetin and kaempferol, as well as several phenolic acids (*p*-hydroxybenzoic acid, gallic acid, ferulic acid and caffeic acid) were identified in all extracts. The highest concentrations of bioactive compounds were detected in the extracts of *T. farfara* (9587.6 μg/mg quercetin and 4875.3 μg/mg caffeic acid) as well as *S. nigra* (4788.8 μg/mg kaempferol).

Conclusions: We can state that the methanolic plant extract of *T. farfara* showed the strongest antimicrobial activity against *Saccharomyces cerevisiae* as well as other tested microorganisms. At the same time, a good antimicrobial activity was found in the other medical plant extracts as well. No antimicrobial effect of the *S. nigra* extract was found with respect to the growth of *Pseudomonas aeruginosa*, *Enterococcus raffinosus* and *Saccharomyces cerevisiae*.

KEYWORDS

Wild medical plants, Antimicrobial activity, Minimum inhibitory concentration, Chemical composition

1. Introduction

The research interest on the production of biologically active compounds from natural resources has been increasing over the past decade[1-9]. Many efforts have been made to discover new antimicrobial substances from different sources such as microorganisms, animals and plants, which have found to be useful in various traditional and folk medicines[10,11]. Natural products of higher plants may provide a variety of antimicrobial agents with possibly novel mechanisms of action[12-15]. Medical plants represent such a rich source of antimicrobial substances, and many

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of them have been already used in different countries as central components of potent and powerful drugs[16]. Furthermore, infections have increased to a great extent in recent years with the downside of antibiotics resistance as an essential therapeutic concern[17]. The extensive use of synthetic drugs as well as unwanted medication, will cause increasing side effects to the body; sometimes, the toxic effects caused by the administration of drugs may be much more serious than the disease itself. In recent years, pharmaceutical companies have invested significantly in testing natural products extracted from plants, to produce more cost effective remedies that are affordable to common people. Plant extracts have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory conditions[18,19]. The selection of crude plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure compounds that are isolated from natural products[20]. The present study was designed to determine the role of methanolic extracts from *Tussilago farfara (T. farfara)*, *Equisetum arvense (E. arvense)*, *Sambucus nigra (S. nigra)* and *Aesculus hippocastanum (A. hippocastanum)* for potential antibacterial and antifungal activity against selected microorganisms, namely, *Escherichia coli (E. coli)*, *Serratia rubidaea (S. rubidaea)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus epidermis (S. epidermis)*, *Lactobacillus rhamnosus (L. rhamnosus)*, *Enterococcus raffinosus (E. raffinosus)* and *Staphylococcus epidermis (S. cerevisiae)*.

2. Materials and methods

2.1. Plant materials

The plant materials used in this experiment consist of flowers from *S. nigra* and *A. hippocastanum*, flowers and stems from *T. farfara*, leaves and stems from *E. arvense*. The plants were collected from Nitra and Gelnica outskirts (Slovakia) during the spring season of 2012. The material was initially dried at the room temperature in the dark. More detailed information is available in Table 1.

Table 1
Detailed information about the plant extracts.

Plants	Plant parts	Yield*	Area
T. farfara	flower + stem	757.6	Gelnica
E. arvense	leaf + stem	392.5	Nitra
S. nigra	flower	442.4	Nitra
A. hippocastanum	flower	509.5	Nitra

*Yield (in mg) from 50 g dried plants per 400 mL methanol.

2.2. Microbial tests

Seven strains of microorganisms were tested in this research, including three Gram-negative bacteria (*E. coli* CCM 3988, *S. rubidaea* CCM 4684, *P. aeruginosa* CCM 1960), three Gram-positive bacteria (*S. epidermis* CCM 4418, *L. rhamnosus* CCM 1828, *E. raffinosus* CCM 4216) and one yeast strain (*S. cerevisiae* CCM 8191). All tested strains were collected from the Czech Collection of Microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37 °C and the yeast suspension was cultured in the malt extract broth (Biomark, India) at 30 °C.

2.3. Preparation of plant extracts

After drying, the plant materials were crushed, weighed out to 50 g and soaked separately in 300 mL of methanol p.a. (99.5%, Sigma, Germany) during two weeks at room temperature. Exposure to sunlight was avoided in order to prevent the degradation of active components. Then, methanolic plant extracts were filtered through the Whatman No. 1 filter paper. The obtained extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the methanol (Stuart RE300DB rotary evaporator, Bibby Scientific Limited, UK, and vacuum pump KNF N838.1.2KT.45.18, KNF, Germany). For the antimicrobial assays, the crude plant extracts were dissolved in dimethyl sulfoxid (DMSO) (Penta, Czech Republic) to 102.4 mg/mL as stock solution, while for chemical analysis methanol was used as solvent. Stock solutions of plant extracts were stored at −16 °C in refrigerator until use.

2.4. Antimicrobial assay

The minimum inhibitory concentration (MIC) is the lowest concentration of the sample that will inhibit the visible growth of microorganisms. Plant extracts dissolved in DMSO were prepared to a final concentration of 1 024 μg/mL by dissolving stock solution with 102.4 mg/100 mL. MICs were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation 2009[21] in Mueller Hinton broth (Biolife, Italy) for bacteria and Sabouraud broth (Biolife, Italy) for yeast. Briefly, the DMSO plant extracts solutions were prepared as serial two-fold dilutions, in order to obtain a final concentration ranging between 0.5-512 μg/mL. Each well was then inoculated with microbial suspension at the final density of 0.5 McFarland. After 24 h incubation at 37 °C for bacteria and 30 °C for yeast, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The 96 micro-well plates were measured before and after experiment. Differences between both measurements were evaluated as growth. Measurement error was established for 0.05 values from absorbance. Wells without plant extracts were used as positive controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the MICs of used medical plant extracts.