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SCREENING OF PLANT EXTRACTS FOR ANTIMICROBIAL ACTIVITY AGAINST BACTERIA

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ABSTRACT

The aim of this study was antimicrobial action of the methanolic extracts of *Equisetum arvense* L. and *Urtica dioica* L. against gramnegative and grampositive bacteria. The antimicrobial activities of the extracts against 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 were determined by the disc diffusion method and the microbroth dilution method 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 most antimicrobial activity showed methanolic plant extract of *E. arvense* against *S. epidermis* with disc diffusion method and with microbroth dilution method against S. *rubidaea* and plant extract *Urtica dioica* with disc diffusion method against *P. aeruginosa* and with microbroth dilution method against *S. rubidaea* and *E. coli*.

Keywords: *Equisetum arvense* L., *Urtica dioica* L., metnanolic extracts, gramnegative and grampositive bacteria

INTRODUCTION

In recent years, the usage of plant materials as food supplement and as alternative medicine has increased due to their phytochemical contents. Among these phytochemicals, alkaloids, carotenoids and phenolics have been widely studied. The most popular area in research is the antioxidant capacities of these substances. Phenolic compounds present in plants exhibit strong antioxidant activities (**Guimaraes *et al.*, 2009; Barros *et al.*, 2010**).
Equisetum arvense L. (field horsetail) is a fern from the *Equisetaceae* family, widely spread across the northern hemisphere as a weed in fields and uncultivated land. Multiple healthfulness properties of field horsetail have been known since ancient times and it has been used in the treatment of pulmonary tuberculosis and haemorrhage, anaemia, peptic and other types of ulcers, fistulas and colon polyps, inflammation, bleeding, kidney and bladder tuberculosis (**Sandhu *et al.*, 2010; Labun *et al.*, 2013**). A large number of papers verify various biological effects of the *E. arvense* extracts, such as sedative and anticonvulsive, hepatoprotective, antioxidant, antibacterial and antifungal activity (**Hyuncheol *et al.*, 2004; Dos Santos *et al.*, 2005; Stajner *et al.*, 2006; Canadanovic-Brunet *et al.*, 2009; Garcia *et al.*, 2011**). *E. arvense* is well-known for its high content of bioactive components, such as: phenolic compounds, saponins, aconite, oxalic and malic acid, resins, tannins, pectin, flavonic compounds, vitamin C, carotenoids and mineral substances (**Radulovic *et al.*, 2006; Uslu *et al.*, 2013**). In 2006 researchers investigated the composition and antimicrobial properties of essential oils from equisetum arvense. The twenty five compounds with antimicrobial activities were identified in the essential oil obtained from the aerial parts of the plant (**Radulovic et al., 2006**). In 2009 a report was published about the antimicrobial and hydroxyl radical scavenging activities of methanol extract of the aerial parts of the plant (**Canadanovic-Brunet *et al.*, 2009**). Antitumoral activities of the *Equisetum arvense* peptides were also investigated (**Alexandru *et al.*, 2006**).
The resistant properties of essences have been known from ancient areas and todays, medicinal plants are very valuable in the industry and scientific researches because of their antimicrobial and antioxidant activities (**Singh *et al.*, 2006**). *Urtica dioica* which is a member of *Urticaceae* class, its Latin name is Nettle, has many important functions in traditional treatment because it has a lot of curable effects. There are many reports which show this plant is very effective in the treatment of blood pressure, diabetes, and prostate hyperplasia, rheumatoid arthritis and allergic rhinitis (**Fathi-Azad *et al.*, 2005**). Antimicrobial activities of alcoholic and aqueous extracts of the separate parts of *Urtica* were investigated on the *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans* in the Islamic Azad University Science-Research Tehran. Its summary illustrated that alcoholic extract of *Urtica* seed had the greatest influence on the gram positive bacteria; leaves extract had the maximum effect on the gram negative bacteria, its blossom oil had the highest impact on the antifungal attribute and aqueous essence had positive effect on the all bacteria except *Pseudomonas* (**Majd *et al.*, 2001**).
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**).
Both of these plants are well known in the traditional medicine and their history is very long. In this work, antibacterial activity of *Equisetum arvense* L. and *Urtica dioica* L. leaves against 5 different grampositive and gramnegative bacteria was studied.

MATERIAL AND METHODS

Preparation of crude extracts

Leaves and steams samples of *Equisetum arvense* L. and *Urtica dioica* L. 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 methanol 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 show shows tab. 1.

Table 1 Detail information about plants and plant extracts

Orig. Latin title	Plant parts	Yield	Area	Dissolving time	Extracted by
Urtica dioica	leaf + stem	2007	Nitra	2 weeks at room temperature	Vacuum evaporator from methanol at room temperature at -800 mbar
Equisetum arvense	leaf + stem	392.5	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 microtitre 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

Natural products are considered an important source of new antibacterial agents. Medicinal plants continue to be used world-wide for the treatment of various diseases and have a great potential for providing novel drug leads with novel mechanism of action (**Singh *et al*., 2012**).
The inhibition zone diameter of selected bacteria compared with *E. arvense* illustrated (tab. 2) that this extract had the highest activity against *S. epidermis* only. Whereas, the maximum diameter of inhibition growth *U. dioica* was related against *P. aeruginosa* and slightly against *E. coli, B. thermospacta, L. rhamnosus* and *P. larvae*.
In this study of **Gülçin *et al*. (2004)**, nine different microbial and one yeast species were used to screen the possible antimicrobial activity of water exctract of *U. dioica*. Water extract of *U. dioica* exhibited antimicrobial activity against all tested microorganisms. Of the species used, *Staphylococcus aureus* is one of the most common Gram-positive bacteria causing food poisoning. Its source is not the food itself, but the humans who contaminate food after it has been processed. *Escherichia coli*, belonging to the normal flora of humans, is a Gram-negative bacterium. However, an enterohemmoragic strain of *Escherichia coli* has caused serious cases of food poisoning and preservatives to eliminate its growth are needed.
The results of **Chahardehi *et al*., (2012)** revealed that ethyl acetate, hexane and chloroform extracts showed higher antimicrobial activity than the other crude extracts, where the ethyl acetate extract showed highest inhibition against *B. cereus*, methicillin resistant *Staphyllococus aureus* and *Vibrio parahaemolyticus*. Terpens and phenols of *U. dioica* are one of the major groups associated with the inhibition of microbial infections and cancer (**Dar *et al*., 2012**). *U. dioica* is a rich source of phytochemicals such as phenolic compounds and minerals which can be used as a potential source of useful drugs (**Ahmed *et al*., 2012**).

Table 2 Antibacterial activity of medicinal plants against bacteria in mm

Microorganism	Medicinal plant extract	Mean (mm)
***E. coli* CCM 3988**	**control**	0
	Equisetum arvense	0
	Urtica dioica	5.00
***P. aeruginosa* CCM 1960**	**control**	0
	Equisetum arvense	0
	Urtica dioica	8.00
***Seratia rubidae* CCM 4684**	**control**	0
	Equisetum arvense	0
	Urtica dioica	0
***Listeria ivanovii* CCM 5884**	**control**	0
	Equisetum arvense	0
	Urtica dioica	0
***Listeria innocua* CCM 4030**	**control**	0
	Equisetum arvense	0
	Urtica dioica	0
***E. raffinosus* CCM 4216**	**control**	0
	Equisetum arvense	0
	Urtica dioica	0
***B. thermospacta* CCM 4769**	**control**	0
	Equisetum arvense	0
	Urtica dioica	2.6
***S. epidermis* CCM 4418**	**control**	0
	Equisetum arvense	3.3
	Urtica dioica	0
***L. rhamnosus* CCM 1828**	**control**	0
	Equisetum arvense	0
	Urtica dioica	2.3
***P. larvae* CCM 4483**	**control**	0
	Equisetum arvense	0
	Urtica dioica	4.6

The determination of the MIC by means of the microbroth dilution method (Table 3) showed that plant extract tested exhibited an antimicrobial effect against some of the ten tested microorganisms. The results of the bioassays showed that extract exhibited moderate to appreciable antibacterial activities against all bacteria. However, this activity varies with the kind of bacteria. The best antimicrobial activity at both medicinal plants were found against four gramnegative bacteria.

Table 3 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})			
		Urtica dioica		*Equisetum arvense*	
		MIC 50	MIC 90	MIC 50	MIC 90
Gramnegative microorganisms					
Liv	*Listeria ivanovii* CCM 5884	1.50	1.63	31.71	55.81
Sr	*Serratia rubidaea* CCM 4684	0.75	0.82	24	25.76
Lin	*Listeria innocua* CCM 4030	6.0	6.48	766.01	814.26
Ec	*Escherichia coli* CCM 3988	0.75	0.82	> 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, $p< 0.05$)

The essential oil of *Equisetum arvense* L. in study of **Radulovič *et al*. (2006)** was shown to possess a broad spectrum of strong antimicrobial activity against all tested strains. The diameters of growth inhibition zones ranged from 23 to 37 mm (including the diameter of the disk, 12.7 mm) with the highest inhibition zone values observed against Gram-negative *S. enteritidis* (35 mm) and *K. pneumoniae* (37 mm). Significant reductions in bacterial growth were obtained with medically important pathogens such as *S. aureus* (28 mm). The activity was greater or similar to conventional antibiotics even in the case of *C. albicans* and the fungal filamentous organism *A. niger*. In the present study, the Gramnegative bacteria *K. pneumoniae*, *P. aeruginosa* and *S. enteritidis* were more susceptible than the Grampositive *S. aureus* except for the strains of *E. coli* (Gramnegative) that were the most resistant of the tested bacteria. It has been frequently reported that Gramnegative bacteria are more resistant to the inhibitory effects of essential oils (**Smith-Palmer *et al*., 1998**), and this was attributed to the microbial cell impermeability due to the presence of certain lipopolysaccharides in the cell walls. All tested microorganisms were completely non-susceptible to control disks imbued with ether. The antimicrobial nature of the *E. arvense* essential oil can be attributed to the presence of various substances, mainly the phenolic monoterpene thymol (**Tepe *et al*., 2004; Pattnaik *et al*., 1997**). In addition the combination of thymol and 1,8-cineole may have resulted in a significant synergistic antifungal effect as previously published (**Pina-Vaz *et al*., 2004**). Linalool has also been reported as having antibacterial (**Onawunmi *et al*., 1984**) and antifungal activity (**Reuveni *et al*., 1984**). β-Ionone was shown to possess antimicrobial effect on the strains of *S. aureus* while in the same study β-caryophyllene had no pronounced activity (**Kubo *et al*., 1992**). Very similar patterns of activity of α- and β-ionones compared with the activity of *E. arvense* oil against *P. aeruginosa*, *E. coli*, *S. aureus* and *C. albicans* were observed previously and their activity correlated with their solubility in water, due to the great importance of the diffusion ability of the compounds in the disk diffusion assay (**Griffin *et al*., 1999**). *Trans*-Phytol was the principal active component responsible for the antimycobacterial activity of the methanol extract of *Leucas volkensii* (**Rajab *et al*., 1998**). The essential oil of *E. arvense* showed strong antimicrobial activity *in vitro* and may, despite the small yield, contribute to the medicinal properties of the plant.

CONCLUSION

In conclusion, we can to state that the most antimicrobial activity showed methanolic plant extract of *E. arvense* against *S. epidermis* with disc diffusion method and with microbroth dilution method against S. *rubidaea* and plant extract *Urtica dioica* with disc diffusion method against *P. aeruginosa* and with microbroth dilution method against *S. rubidaea* and *E. coli*.

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