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Original article

Antimicrobial activity of some plant extracts against bacterial pathogens isolated from faeces of red deer (*Cervus elaphus*)

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Abstract

Antibacterial activity is the most widely studied aspect of plant extracts. Antibiotics extensively produced and consumed in large quantities, have proved to be problematic due to various types of adverse effects. The development of bacterial resistance to currently available antibiotics has necessitated the search for new antibacterial agents. One of the alternative strategies for fighting antibiotic-resistant bacteria is the use of natural antimicrobial substances such as plant extracts. We tested the antimicrobial activity of nine extracts from different plants against pathogenic bacteria isolated from the faeces of red deer (*Cervus elaphus*). Selected bacteria commonly contaminated the natural environment and constitute a source of infection in other animals and humans. Extracts obtained from the following plants were tested: *Hypericum perforatum* L., *Chamomilla recutita* L., *Achillea millefolium* L., *Salvia officinalis* L., *Thymus vulgaris* L., *Pinus sylvestris* L., *Mentha x piperita* L., *Valeriana officinalis* L. and *Foeniculum vulgare* Mill. The highest degree of antibacterial properties was observed for *Mentha x piperita* L., narrower spectrum of activity possessed *Hypericum perforatum* L. Extracts of *Achillea millefolium* L. had the lowest spectrum of antibacterial activity. Our study confirms that many plant extracts shows *in vitro* antibacterial activity.

Key words: plant extracts, *Cervus elaphus*, antibacterial activity, antibiotic-resistant bacteria

Introduction

Plant extracts are an interesting group of natural products used in medicine due to their wide range of biological activity. New therapeutics often are modelled on plant-derived antibacterials, which have been used in medicine for centuries, although their use has been very limited for some time, and even eliminated by synthetic drugs (Aarestrup 2005, Hemaiswarya et al. 2008). Antibiotics, whose chemical structure has been more thoroughly tested and which are safer to use, extensively produced and consumed in large quantities, have proved to be problematic due to various types of adverse effects (Hemaiswarya et al. 2008). Although antibiotics have been effective in treating infectious diseases, bacterial drug resistance has led to the emergence of new infectious diseases and the re-emergence of old ones (Harada et al. 2012). The development of antimicrobial resistance in bacteria of animal origin reduces the efficacy of veterinary antimicrobial drugs (Aarestrup 2005). The effect on human health has been a concern since Swann et al. (1969) reported that antimicrobial-resistant bacteria, arising from the use of veterinary antimicrobial agents, were transmitted to humans through livestock products and reduced the efficacy of antimicrobial drugs in humans.

One of the alternative strategies for fighting antibiotic-resistant bacteria is the use of natural antimicrobial substances such as plant extracts and their components. Current scientific data indicate that synergy and a similar mechanism of action is observed in synthetic drugs and natural products, including flavonoids and essential oils, in effectively combating bacteria (Hemaiswarya et al. 2008, Han et al. 2012, Chen et al. 2013). Additionally, various extracts from plants are widely known as natural antioxidants. The successful use of combinations of plant extracts is not only observed in anti-infective therapy, but also seen in the treatment of illness including cancer, inflammatory states, osteoarthritis and hypertension (Williamson and Manach 2005). The recent trend has been the “herbal shotgun” method, similar to Ayurveda, involving simultaneous application of herbs and antibiotics with similar activity (Hemaiswarya et al. 2008). The natural components in herbal medicaments are as effective as synthetic drugs without causing the emergence of drug-resistant mutants, weakening of the immune system or other side effects (Hemaiswarya et al. 2008, Bhat-tacharjee and Islam 2014).

The flavonoids present in many plants have beneficial effects on animal health. Their activity consists in reduce amino acid oxidation, exert an antimicrobial effect against some intestinal microorganisms,

promote intestinal absorption, stimulate enzyme secretion, increase feed palatability (Ruberto and Baratta 2000, Avato et al. 2004, Medhat et al. 2013, Singh et al. 2015). Thereby flavonoids stimulating intake, and improve the immune status of the animal (Hemaiswarya et al. 2008, Vivek et al. 2009, Saddiqe et al. 2010, Medhat et al. 2013, Singh et al. 2015).

Peppermint extracts contain essential oil with bioactive compounds such as menthol, menthone, menthyl acetate, menthofuran and limonene (Diaz et al. 1988, Singh et al. 2015). They present antibacterial, antifungal, antiviral and antioxidant activity (Chaumont et al. 1978, Diaz et al. 1988, Singh et al. 2015). Peppermint oil is mostly used in the symptomatic treatment of digestive disorders such as dyspepsia, flatulence and gastritis (Bupesh et al. 2007). Strong biological properties of common Saint John’s wort are linked to its high content of flavonoids: apigenin, quercetin, biapigenin, amentoflavone, hypericin and hyperforin (Barnes et al. 2002, Avato et al. 2004, Orhan et al. 2013). Pharmacological studies have demonstrated that common Saint John’s wort extract has sedative and anti-inflammatory properties, and present antiviral, and antifungal activity (Barnes et al. 2002). *In vitro* and *in vivo* studies have confirmed that extracts of this plant can be applied as a natural wound-healing remedy (Barnes et al. 2002, Avato et al. 2004), which is partially linked to its antibacterial activity. The antiseptic, antioxidant, anaesthetic, insecticidal and food-preservative properties of common thyme are due to the presents of biologically active volatile compounds (Hudaib et al. 2002, Medhat et al. 2013).

The aim of the study was to determine the susceptibility of selected bacterial strains to extracts obtained from plants naturally occurring in Poland. To evaluate the level of susceptibility of strains tested, the resistance profiles were determined to 17 antibacterials commonly used in veterinary medicine.

Materials and Methods

Plant extracts and cultures

Extracts obtained from the following plants were tested: common Saint John’s wort (*Hypericum perforatum* L.), chamomile (*Chamomilla recutita* L.), common yarrow (*Achillea millefolium* L.), common sage (*Salvia officinalis* L.), common thyme (*Thymus vulgaris* L.), Scots pine (*Pinus sylvestris* L.), peppermint (*Mentha x piperita* L.), valerian (*Valeriana officinalis* L.) and fennel (*Foeniculum vulgare* Mill.). The plants were collected from May to October 2014 from forests in Poland (the Kluczbork Forest District, the Stob-

rawskie Forest). The systematic identification of the plant materials was verified (Jesionek et al. 2013). Plant materials were stored in a cool and dry place until further analysis.

The bacterial strains used in the study, i. e. *Escherichia coli* EC-ZMW, *Klebsiella pneumoniae* KP-ZMW, *Yersinia enterocolitica* YC-ZMW, *Staphylococcus aureus* SA-ZMW, *Listeria monocytogenes* LM-ZMW and *Enterococcus faecalis* EC-ZMW (10 isolates of each species were used in the study, marked with the numbers of the Department of Veterinary Microbiology collection from 1 to 10). The bacteria came from screening tests identifying the microflora of red deer (*Cervus elaphus*) faeces (Gnat et al. 2015). Strains from the American Type Culture Collection (ATCC), i. e. *E. coli* ATCC25922, *K. pneumoniae* ATCC13883, *Y. enterocolitica* ATCC23715, *S. aureus* ATCC25923, *L. monocytogenes* ATCC35152, and *E. faecalis* ATCC29212, were used as reference standards for the plant extracts testing. All bacterial isolates were subcultured on Brain Heart Infusion agar (BHI, Biocorp).

Extracts preparation

Tinctures of common Saint John's wort, chamomile, common yarrow, common thyme and common sage were obtained by seven-day maceration of aerial parts with 70% ethanol (v/v). The valerian tincture was prepared by seven-day maceration of roots with 70% ethanol (v/v). The peppermint tincture contained 5% peppermint essential oil and 95% peppermint tincture obtained by one-day maceration of peppermint leaves with 95% ethanol (v/v). The Scots pine tincture was obtained by five-day percolation of tree branches with 90% ethanol (v/v). The fennel tincture was prepared by seven-day maceration of seeds with 70% ethanol (v/v). The selection of extraction solvent and sample preparation procedures were based on *Polish Pharmacopoeia VIII*. The plant extracts were stored at 4°C in dark glass bottles to prevent degradation by light and temperature.

Extracts Disc Diffusion Method

Extracts were screened for antibacterial activity by the disc diffusion method, using an 18 h culture at 37°C in 10 ml of Mueller Hinton Broth (Oxoid). The bacterial cultures were diluted with sterile physiological saline solution (PS, 0.85% sodium chloride) with reference to the McFarland standard (bioMerieux) to achieve an inoculum of approximately 10^6 CFU ml⁻¹. One hundred microlitres of the suspensions were

placed on plates with Mueller Hinton Agar (Oxoid) to obtain uniform microbial growth on the plates. Blank discs (Oxoid) were placed on the agar surface and 50 µl of prepared extracts was spotted on each disc. The solvent ethanol was placed on the discs as a control. The plates were left for 30 min at room temperature to allow diffusion of the extracts and then incubated at 37°C for 24 h. After this time, to facilitate visualization of bacterial growth, thiazolyl blue tetrazolium bromide (8 mg/10 ml H₂O) was applied to the plate, and the zones of inhibition were measured in millimetres. The experiments were performed in triplicate and the mean value was calculated.

Extracts Minimal Inhibitory Concentrations

The minimum inhibitory concentration (MIC) method was used for evaluation of antimicrobial activity of EOs. The serial, two-fold dilution of each plant extract was prepared over 6.25 – 0.025%. The broth was supplemented with Tween 80 (Sigma Aldrich Germany) at a concentration of 0.5% to enhance EOs solubility. One hundred microliters of each dilution was distributed in a microtiter 96-well plates, as well as sterility and growth controls (with and without Tween 80) were prepared. Twenty four-hours broth cultures of each strain were prepared in nutrient broth (Biocorp, Warsaw, Poland). The cultures of bacteria were diluted to obtain the final concentration of 5×10^5 CFU/ml. The plates were incubated aerobically at 37°C for 24 h. The MIC was defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth (compared to the growth of control wells).

Antibiotic Disc Diffusion Method

Antimicrobial susceptibility testing was performed in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI). The disc diffusion method was used to determine bacterial susceptibility to 17 antimicrobial agents (Oxoid): cefoxitin (FOX, 30 µg), erythromycin (E, 15 µg), clindamycin (CD, 2 µg), tetracycline (TE, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), rifampicin (Rd, 5 µg), gentamicin (CN, 10; 120 µg), trimethoprim sulfamethoxazole (SXT, 25 µg), amoxicillin with clavulanate (AMC, 30 µg), ertapenem (ETP, 10 µg), kanamycin (K, 30 µg), ampicillin (AMP, 10 µg), cefuroxime (CXM, 30 µg), oxacillin (OX, 1 µg), vancomycin (VA, 30 µg) and streptomycin (S, 300 µg). Bacterial isolates were inoculated on Mueller-Hinton Agar (Oxoid).

Table 1. Antimicrobial activity of nine extracts against bacterial isolates from red deer faeces and reference strains determined by the disc diffusion method.

Plant extracts	Bacterial strains (isolates and reference strains)											
	<i>Escherichia coli</i> EC-ZMW 1-10	<i>Escherichia coli</i> ATCC25922	<i>Klebsiella pneumoniae</i> KP-ZMW 1-10	<i>Klebsiella pneumoniae</i> ATCC13883	<i>Yersinia enterocolitica</i> YC-ZMW 1-10	<i>Yersinia enterocolitica</i> ATCC23715	<i>Staphylococcus aureus</i> SA-ZMW 1-10	<i>Staphylococcus aureus</i> ATCC25923	<i>Listeria monocytogenes</i> LM-ZMW 1-10	<i>Listeria monocytogenes</i> ATCC35152	<i>Enterococcus faecalis</i> EF-ZMW 1-10	<i>Enterococcus faecalis</i> ATCC29212
<i>Hypericum perforatum</i> L.	NI	NI	7.0 ± 1.2	NI	NI	NI	10.0 ± 0.2	11.0 ± 0.2	13.3 ± 0.1	12.4 ± 0.2	11.4 ± 0.8	11.0 ± 0.7
<i>Chamomilla recutita</i> L.	NI	7.2 ± 2.1	NI	NI	NI	NI	NI	NI	NI	NI	7.6 ± 0.8	7.4 ± 0.3
<i>Achillea millefolium</i> L.	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	8.0 ± 1.6	8.1 ± 0.5
<i>Thymus vulgaris</i> L.	NI	NI	8.5 ± 0.4	7.3 ± 0.1	NI	NI	10.7 ± 0.3	11.6 ± 0.4	10.2 ± 0.5	9.0 ± 0.2	8.8 ± 1.2	8.1 ± 1.1
<i>Salvia officinalis</i> L.	NI	NI	7.0 ± 0.1	NI	NI	NI	12.1 ± 0.4	12.5 ± 0.2	12.4 ± 0.3	12.0 ± 0.1	10.1 ± 0.6	9.0 ± 1.1
<i>Mentha x piperita</i> L.	8.8 ± 0.1	10.6 ± 0.2	9.6 ± 0.2	11.1 ± 0.3	11.8 ± 0.4	11.7 ± 0.5	10.5 ± 0.4	12.5 ± 0.3	10.6 ± 0.2	8.4 ± 0.4	7.9 ± 1.3	8.6 ± 0.8
<i>Pinus sylvestris</i> L.	8.0 ± 0.4	7.6 ± 0.2	7.1 ± 0.2	NI	NI	NI	NI	8.5 ± 0.1	9.0 ± 1.2	8.6 ± 0.8	8.4 ± 0.6	8.4 ± 0.8
<i>Valeriana officinalis</i> L.	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	7.3 ± 0.5	7.3 ± 0.9
<i>Foeniculum vulgare</i> Mill.	7.0 ± 0.5	7.6 ± 0.3	7.6 ± 0.8	7.5 ± 1.2	NI	NI	NI	NI	7.4 ± 0.6	7.4 ± 0.8	7.8 ± 0.8	7.8 ± 0.8

Legend: Zones of growth inhibition (mm) showing antibacterial activity for selected plant extracts, NI – no inhibition, ± SEM
Source of bacterial strains: ATCC (American Type Culture Collection) and ZMW (Department of Veterinary Microbiology, University of Life Sciences in Lublin, Poland).

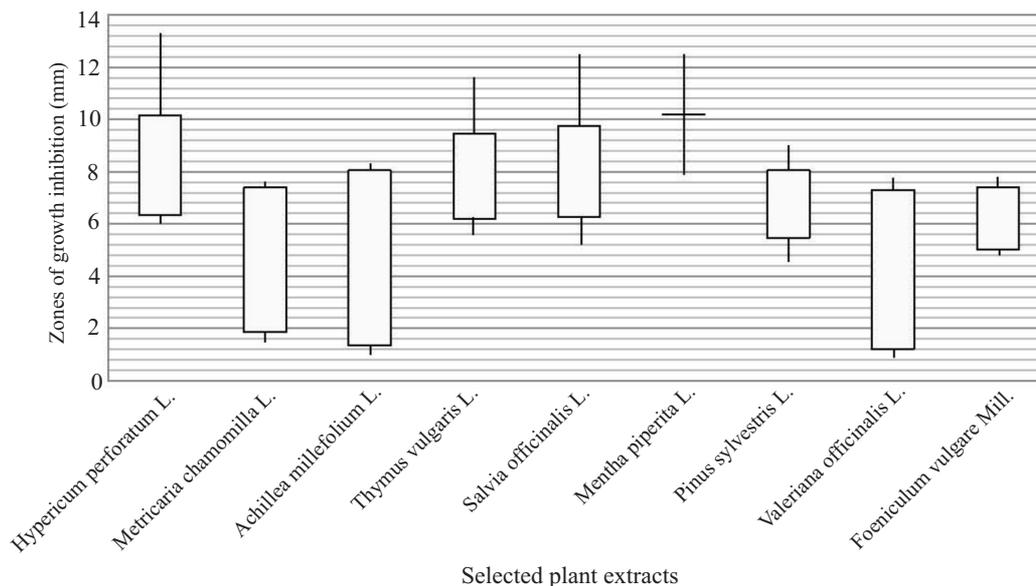


Fig. 1. Relative effectiveness of the antimicrobial activity of extracts from different plants.

Figure legend: The rectangles represent zones of inhibition in a 95% confidence interval for all examined microorganisms; the relative effectiveness of the use of an essential oil is considered statistically significant ($p < 0.5$). Vertical bars indicate the maximum deviation of the results.

Statistical analysis

Results were expressed as mean value with standard error of the mean (SEM) of growth inhibition zones diameters. In order to assess the effectiveness of the antibacterial effect of the test plant extracts was used confidence intervals (CI; 95%) calculated values of the T-test for all examined microorganisms. The results are presented in the form of a graph with an indication of the confidence intervals between the borders of the rectangle, in addition to the extreme zones of inhibition were determined lines. Student's T-test was used to compare results between MIC values obtained for each plant extract. To the analysis was been used the R program.

Results

The results of the antibacterial screening of samples of plant extracts are presented in Table 1. Both the isolates and the reference strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Yersinia enterocolitica* were susceptible to the effect of the oils contained in the peppermint extract. The mean values for the zones of growth inhibition for these bacteria ranged from 8.8 to 11.7 mm. Furthermore, *Klebsiella pneumoniae* strains were susceptible to the common thyme oils (growth inhibition zone 7.3-8.5 mm), and like *Escherichia coli*, to the fennel oils (growth inhibition zone 7.0-7.6 mm).

Staphylococcus aureus, *Listeria monocytogenes* and *Enterococcus faecalis* also exhibited susceptibility to peppermint oils (growth inhibition zone 8.4-12.5 mm), and to the oils of common Saint John's wort (10.0-13.3 mm), common thyme (8.1-11.6 mm) and common sage (9.0-12.5 mm), respectively. *Enterococcus faecalis* was found to be the most sensitive to the effect of the extracts tested and was the only bacterial species that was also susceptible to the valerian oils (growth inhibition zone 7.3 mm) (Table 1).

The broadest antibacterial activity was noted for the peppermint extract (growth inhibition for all the microorganisms tested were 10.1 mm, within a 95% confidence interval ($p < 0.5$) (Fig. 1). At the other extreme, with the most limited antibacterial activity, were the extracts of common yarrow, with zones of growth inhibition ranging from 1.5 to 8.1 mm (95%, $p < 0.5$), respectively. Similar and relatively strong antibacterial effects were observed for the extracts from common Saint John's wort (growth inhibition zone 6.4-10.1), common thyme (6.2-9.3), common sage (6.6-9.7), Scots pine (5.6-8.0) and fennel (7.0-7.6 mm, Fig. 1), for which the ranges of the zones of growth inhibition (95%, $p < 0.5$).

In the case of MIC determination, the results obtained were consistent with the ranges of growth inhibition zones in the disc diffusion method. The strongest antimicrobial effect was found in the extract of peppermint, growth of bacteria was inhibited by 0.1 – 1.56% peppermint extracts (Table 2). The weakest antimicrobial effect has been demonstrated for com-

Table 2. MICs values (%) of nine extracts against bacterial isolates from red deer faeces and reference strains.

Plant extracts	Bacterial strains (isolates and reference strains)											
	<i>Escherichia coli</i> EC-ZMW 1-10	<i>Escherichia coli</i> ATCC25922	<i>Klebsiella pneumoniae</i> KP-ZMW 1-10	<i>Klebsiella pneumoniae</i> ATCC13883	<i>Yersinia enterocolitica</i> YC-ZMW 1-10	<i>Yersinia enterocolitica</i> ATCC23715	<i>Staphylococcus aureus</i> SA-ZMW 1-10	<i>Staphylococcus aureus</i> ATCC25923	<i>Listeria monocytogenes</i> LM-ZMW 1-10	<i>Listeria monocytogenes</i> ATCC35152	<i>Enterococcus faecalis</i> EF-ZMW 1-10	<i>Enterococcus faecalis</i> ATCC29212
<i>Hypericum perforatum</i> L.	>6.25	>6.25	6.25	>6.25	>6.25	>6.25	0.39	0.39	0.2	0.2	0.39	0.39
<i>Chamomilla recutita</i> L.	>6.25	3.12	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	1.56	1.56
<i>Achillea millefolium</i> L.	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	0.78	0.78
<i>Thymus vulgaris</i> L.	>6.25	>6.25	3.12	6.25	>6.25	>6.25	0.2	0.2	0.39	0.39	0.78	0.78
<i>Salvia officinalis</i> L.	>6.25	>6.25	3.12	>6.25	>6.25	>6.25	0.1	0.1	0.1	0.1	0.2	0.39
<i>Mentha x piperita</i> L.	0.39	0.2	0.39	0.2	0.2	0.2	0.1	0.2	0.2	1.56	1.56	0.78
<i>Pinus sylvestris</i> L.	0.78	3.12	3.12	>6.25	>6.25	>6.25	>6.25	0.78	0.78	0.78	1.56	1.56
<i>Valeriana officinalis</i> L.	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	1.56	1.56
<i>Foeniculum vulgare</i> Mill.	3.12	1.56	1.56	3.12	>6.25	>6.25	>6.25	>6.25	1.56	1.56	1.56	1.56

Legend: Source of bacterial strains: ATCC (American Type Culture Collection) and ZMW (Department of Veterinary Microbiology, University of Life Sciences in Lublin, Poland)

Table 3. Antibiotic susceptibility (in mm) of strains isolated from faeces of red deer.

	<i>Escherichia coli</i> EC-ZMW		<i>Yersinia enterocolitica</i> YC-ZMW		<i>Klebsiella pneumoniae</i> KP-ZMW		<i>Staphylococcus aureus</i> SA-ZMW		<i>Listeria monocytogenes</i> LM-ZMW		<i>Enterococcus faecalis</i> EF-ZMW	
AMC30	19 ± 0.4	R	13 ± 0.4	S	27 ± 1.2	R	NT		27 ± 2.2	R	NT	
AMP10	20 ± 1.4	R	29 ± 1.2	R	24 ± 1.6	R	37 ± 2.2	R	NT		18 ± 0.9	I
C30	27 ± 0.5	R	29 ± 1.5	R	0	S	NT		NT		17 ± 0.4	I
CD2	NT		NT		NT		22 ± 0.8	R	10 ± 1.8	S	NT	
CIP10	47 ± 2.5	R	42 ± 3.5	R	18 ± 0.4	I	33 ± 3.8	R	28 ± 1.5	R	17 ± 0.4	I
CN10	26 ± 1	R	42 ± 3	R	16 ± 0.5	R	21 ± 1.2	R	NT		NT	
CN120	NT		NT		NT		NT		NT		19 ± 2.7	R
CXM30	26 ± 1.5	R	18 ± 0.3	R	17 ± 0.2	I	NT		NT		NT	
E15	NT		NT		NT		21 ± 1	I	27 ± 2	R	16 ± 0.3	I
ETP10	35 ± 0.3	R	13 ± 0.2	S	18 ± 0.4	I	NT		NT		NT	
FOX30	28 ± 1.2	R	34 ± 1.4	R	25 ± 1.4	R	24 ± 1	R	NT		NT	
K30	24 ± 0.9	R	27 ± 2	R	28 ± 0.9	R	NT		NT		NT	
OX1	NT		NT		NT		23 ± 0.8	R	NT		NT	
RD5	NT		NT		NT		NT		27 ± 2.5	R	NT	
S300	NT		NT		NT		NT		NT		16 ± 1.5	R
SXT25	NT		NT		NT		NT		0	S	NT	
TE30	30 ± 1.2	R	25 ± 1	R	23 ± 1	R	31 ± 1.5	R	24 ± 1.4	R	18 ± 0.2	I
VA30	NT		NT		NT		16 ± 0.4	R	NT		16 ± 0.4	I

Legend: AMC30 – amoxicillin+clavulanate; ETP10 – ertapenem; K30 -kanamycin; CN10 – gentamicin; CN120 – gentamicin; AMP10 – ampicillin; C30 – chloramphenicol; TE30 – tetracycline; CIP10 – ciprofloxacin; CXM30 – cefuroxime; FOX30 – ceftiofur; OX1 – oxacillin; VA30 – vancomycin; CD2 – clindamycin; E15 – erythromycin; RD5 – rifampicin; SXT25 – sulfamethoxazole; S300 – streptomycin; NT – not tested; R – resistant; I – intermediate; S – susceptible. The number next to the name of the antibiotic is the concentration in µg/ml.

mon yarrow, valerian and chamomile extracts, growth inhibitory effect was obtained for *Enterococcus faecalis* at 0.78, 1.56 and 1.56% extracts, respectively. In the case of 3.12% of the chamomile extract, the reference strain *Escherichia coli* ATCC25922 also showed a sensitivity. The remaining MIC values of the tested extracts were in the range of 0.1 – 3.12%, the differences were not statistically significant.

Evaluation of the susceptibility of the isolated bacteria to the antibiotic shows that the *Escherichia coli* and *Staphylococcus aureus* isolates were resistant to all the antibiotics tested. The *Yersinia enterocolitica* strains exhibited susceptibility to amoxicillin with clavulanic acid and to ertapenem, *Klebsiella pneumoniae* to chloramphenicol, and *Listeria monocytogenes* to clindamycin and sulfamethoxazole. The *Enterococcus* isolates reacted with intermediate sensitivity to most of the antibiotics used except gentamicin in which case it was resistant (Table 3).

Discussion

In this study, the antimicrobial activity of nine extracts from different plants often used in phytotherapy were tested against potentially pathogenic bacteria (Table 1). The bacteria identified

in our study were isolated from the faeces of red deer (*Cervus elaphus*), which contaminate the natural ecosystem and may be a source of infection in other animals and humans. Recently, the antimicrobial activity of plant oils and extracts has become the basis for numerous applications, including alternative and natural medicines (Cowan 1999, Burt 2004) and they represent sources of biologically active organic compounds with high potential for treating infectious diseases of bacterial, viral or fungal etiology (Brantner and Grein 1994, Lewis and Elvis-Lewis 1995, Yan-kauer 1997, Helander et al. 1998, Cowan 1999, Ultee et al. 1999, Cox et al. 2000, Delaguis et al. 2002).

Epidemiologically the most dangerous infectious agent isolated from the red deer faeces was *Escherichia coli*, that can grow in fresh or sterile water at low carbon source concentration, and has been implicated as the origin of number of human and animal diseases (Vital et al. 2008, Dobrovsky et al. 2013). Another bacteria species important in terms of the risk it poses to the health of humans and animals is *Yersinia enterocolitica* (Table 1), which is widespread in soil, water, and pastureland. Wild animals, including deer become infected by eating or drinking contaminated material, and shed the bacteria in their faeces (Slee and Skilbeck 1992). *Listeria monocytogenes* is frequently isolated in agriculture areas

and considered to be one of the main cause of mortality in deer populations (Tham 1999). It is also sporadically present in aquatic environments, where may pose an epizootic threat (Lis-Balchin and Deans 1997). According to Botzler et al. 1973 and Nowakiewicz et al. (2015), who isolated *Listeria monocytogenes* from turtle faeces, the use of the same water bodies by animals can lead to infections.

In our study, we found that the microorganisms tested were susceptible to plant extracts, and the highest degree of antibacterial properties was observed for peppermint extract, which was active against all the investigated bacteria. These observations are consistent with earlier studies carried out by Bupesh et al. (2007), Sujana et al. (2013), and Singh et al. (2015). Additionally, Singh et al. (2015) indicated that Gram-positive bacterial species (*Staphylococcus aureus*) tested were more sensitive to peppermint essential oil (the inhibition zone 17.2 mm), than Gram-negative bacteria (*Escherichia coli*; the inhibition zone 5.1 mm). Similarly to our study, peppermint ethanol extracts are more effective against Gram-positive and less against Gram-negative bacteria (the inhibition zones for isolated *Staphylococcus aureus* and *Escherichia coli* were 10.5 mm and 8.8 mm, respectively). The weak antibacterial activity against Gram-negative bacteria has been ascribed to the presence of an outer membrane with hydrophilic polysaccharide chains as a barrier to hydrophobic plant extracts (Sikkema et al. 1994, Tassou et al. 1995, Jones 1997, Mann et al. 2008). It seems that the relationship between the structure of bacterial cell wall (Gram-positive and Gram-negative bacteria) and the sensitivity of plant extracts can be generalized for all plants (Nakatani 1994, Burt 2004, Hemaiswarya et al. 2008, Jesionek et al. 2013).

The use of common Saint John's wort in the traditional medicine was confirmed in *in vitro* studies on antibacterial activity of plant extracts of extracts of its aerial parts (Barnes et al. 2002, Avato et al. 2004, Orhan et al. 2013). Mazandarani et al. (2007) reported higher antibacterial activity of ethanolic extracts of flowering aerial parts of common Saint John's wort against Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), with growth-inhibition zones in the range of 25-26 mm, than of Gram-negative bacteria (*Salmonella* Typhi, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Escherichia coli* and *Pseudomonas aeruginosa*), against which no or only minimal activity was observed. This results are consistent with ours, common Saint John's wort extract had a narrower spectrum of activity than peppermint, inhibition has not been observed for Gram-negative bacteria (except *Klebsiella pneumoniae*) but the inhibition zones of three of the four

sensitive strains were over 0.5 – 4.0 mm higher for both isolated and reference bacteria.

The relatively high activity of common thyme in our study, comparable to the peppermint activity (the inhibition zones were 0.2 – 3.8 mm lower and the spectrum was about 30% narrower), is probably linked to the presence of two bioactive isomeric terpenes: thymol and carvacrol (Horvth et al. 2004, Móricz et al. 2012). In the literature, there is no accurate data about the antibacterial effect of thyme extracts and essential oils. Only results obtained by El-Hosseiny et al. (2014) indicated that thyme essential oil, with its high content of the phenolic monoterpene thymol, exhibited the antipseudomonal activity when used singly or combined with conventional antibiotics.

The bioactivity of Scots pine and fennel extracts resulted in smaller inhibition zones. Of all the bacteria tested, the most sensitive was *Enterococcus faecalis* (both the antibiotic and the plant extracts) and the most resistant strain was *Yersinia enterocolitica*.

The definition of MIC given by most of the researchers as a measure of the antibacterial performance of antibiotics but it appears that no standardised test has been developed for MIC values of plant extracts and essential oils (Burt 2004). The CLSI method for antibacterial susceptibility testing, which is principally aimed at the testing of antibiotics was not extended by the guidelines for plant extracts (Hammer et al. 1999). For this reason, the authors often to compare the results of their research uses growth inhibition zones obtained in the disc diffusion method (Sujana et al. 2013, Singh et al. 2015). In our studies, we used percentage dilutions of extracts and the resulting ranges of MIC values were consistent with the growth inhibition zones obtained for the bacteria.

The presence of microorganisms that are resistant to antibiotics and other synthetic antimicrobial medicines in wild animals represents a concern to public health (Slee and Skilbeck 1992, Mazandarani et al. 2007, Dobrovsky et al. 2013, El-Hosseiny et al. 2014, Smith et al. 2014, Nowakiewicz et al. 2015, Singh et al. 2015). Smith et al. (2014) suggest that these resistance bacteria in wild species could result in wildlife hosts functioning as reservoirs of pathogens for human or livestock populations. In our study we have described bacteria resistant to antibiotics, in which the zone of inhibition exceeds the reference range (CLSI 2011). Most of all, *Escherichia coli* which is important from an epidemiological standpoint, and *Staphylococcus aureus* showed resistance to all antibiotics used, according to CLSI standard. Despite such high resistivity, these bacteria have demonstrated sensitivity to three (peppermint, Scots pine, and fennel) and four (common Saint John's wort, common thyme, common

sage, and peppermint) extracts, respectively. Interestingly, *Yersinia enterocolitica* was sensitive only to two antibiotics: amoxicilin with clavulanate and er-tapenem (for remaining eight tested resistance), and has been revealed the inhibition zone for one of the extract obtained from peppermint. Similar results were obtained by Mazandarani et al. (2007), El-Hosseiny et al. (2014), and Singh et al. (2015) which extends a confirmatory application that the plant oils and extracts can be effective in the fight against microorganisms.

In summary, this study confirms that many plant extracts possess *in vitro* antibacterial activity. Effective antimicrobial therapy using the available arsenal of both drugs and plant extracts is crucial for protecting animal and human health into the future. Thus, a complete understanding of the role played by antimicrobial activity of plants in environments occupied by humans and by domestic, domesticated and wild animals is required to provide scientifically tested mitigation measures capable of addressing this public health challenge (Ulrich-Merzenich et al. 2007).

The development of bacterial resistance to currently available antibiotics has necessitated the search for new antibacterial agents (Bhattacharjee and Islam 2014). Due to the resistance that microorganisms have built against current antibiotics, numerous researchers have shown interest in biologically active components isolated from plants and their influence on elimination of pathogenic microorganisms (Tepe et al. 2004). Recent developments in genomics, proteomics and metabolomics have created a new platform to determine the synergistic efficacy of phytoextracts and their mode of action. This may lead to new phyto-based paradigms towards the use of complex plant mixtures in medicine (Ulrich-Merzenich et al. 2007).

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