INTRODUCTION

Plants have several ways of taking up nutrients; among them one of the highly effective way is carnivory. The more than 600 known species of carnivorous plants constitute a very diverse group, often very distantly related species originating from different systematic orders and families. The common feature of those plants is the ability to hunt and consume animals and this unites in this group the species from various climatic and geographical areas differing morphologically and ecologically (Studnička, 2006). Carnivorous plants capture and utilize nutrients of prey, which consists mostly from insects (Darwin, 1875; Juniper et al., 1989). These plants occur in areas such as wetlands, alpine mountain peaks, vulcanic platforms. They are characterized by growth on the sunny areas, the water-rich sites, but also are able to grow in medium poor of nutrients (Jurgens et al., 2012). Plants have elaborated adaptation to prey on and use the nutrients of victim with specialized leaves, the secretion of sticky substances, digestive enzymes and nectar to attract a prey (Thorén et al., 2003). The ability of plants to catch insects was firstly observed in 1759 at Venus flytrap (Dionaea muscipula) by Arthur Dobbs (Studnička, 2006). A more detailed description of carnivory plants described by Charles Darwin (1875) and still represents the fundamental work on insectivorous plants (Darwin, 1875).

The genus Drosera represents a good model of plant evolution and functional adaptation. Importantly, extracts from numerous species of Drosera have been traditionally used for various medicinal purposes (Samaj et al., 1999). The Drosera genus is a natural source of pharmacologically important secondary compounds used as substrates in the production of pharmaceuticals. The most important are naphthoquinones, especially plumagin,7-methyljuglone and flavonoids (Banasiuk et al., 2014). Particular, naphthoquinones are thought to be responsible for therapeutic effects in respiratory diseases including bronchial infections and tuberculosis. The naphthoquinones, and specially plumagin, also inhibits a development of parasitic nematodes and insects (Collantes et al., 2014). The extracts from Drosera which contain naphthoquinones share medical and other valuable properties and the antiviral, antibacterial, antifungal, aphrodisiac, antispasmodic, antiinflammatory, antierosion and anticancer properties. The aim of this study was to evaluate the antibacterial activity of Drosera rotundifolia extracts by the detection of the minimal inhibitory concentration (MIC). The aim of this study was to evaluate the antibacterial activity of Drosera plant extracts by the detection of the minimal inhibitory concentration (MIC). The aim of this study was to evaluate the antibacterial activity of Drosera plant extracts by the detection of the minimal inhibitory concentration (MIC).

Keywords: Drosera rotundifolia, carnivory, minimal inhibitory concentration (MIC), antibacterial activity

MATERIALS AND METHODS

Plant material

Plants of Drosera rotundifolia L. were cultivated in vitro on basal MS medium (DUCHEFA) supplemented with 2 % (w/v) of sucrose and 0.8 % (w/v) of agar (Bobák et al., 1995). The plantlets were cultivated at 20 ± 2 °C with a day length of 16 h under 50 µEm⁻² s⁻¹ light intensity. Plant extracts were isolated from three plants of Drosera rotundifolia L. (S1, S2 and S3) in different time range. The most effective extract with MIC50 value of 17.07 μg.ml⁻¹ was S3, while forMIC90 of 19.05 μg.ml⁻¹ were extracts S2 and S3 exhibiting antimicrobial activity against Bacillus thuringiensis, Clostridium perfringens and Listeria monocytogenes. Extracts S1, S2 showed MIC50 value 25.53 μg.ml⁻¹ for all the microorganism tested, but S3 extract revealed the same antimicrobial activity against Yersinia enterocolitica, Salmonella enterica subsp. enterica (CCM 3807) and Yersinia enterocolitica (CCM 5671). Plant extracts were isolated from three plants of Drosera rotundifolia L. (S1, S2 and S3) in different time range. The most effective extract with MIC50 value of 17.07 μg.ml⁻¹ was S3, while forMIC90 of 19.05 μg.ml⁻¹ were extracts S2 and S3 exhibiting antimicrobial activity against Bacillus thuringiensis, Clostridium perfringens and Listeria monocytogenes. Extracts S1, S2 showed MIC50 value 25.53 μg.ml⁻¹ for all the microorganism tested, but S3 extract revealed the same antimicrobial activity against Yersinia enterocolitica, Salmonella enterica subsp. enterica (CCM 3807) and Yersinia enterocolitica (CCM 5671). Plant extracts were isolated from three plants of Drosera rotundifolia L. (S1, S2 and S3) in different time range. The most effective extract with MIC50 value of 17.07 μg.ml⁻¹ was S3, while forMIC90 of 19.05 μg.ml⁻¹ were extracts S2 and S3 exhibiting antimicrobial activity against Bacillus thuringiensis, Clostridium perfringens and Listeria monocytogenes. Extracts S1, S2 showed MIC50 value 25.53 μg.ml⁻¹ for all the microorganism tested, but S3 extract revealed the same antimicrobial activity against Yersinia enterocolitica, Salmonella enterica subsp. enterica (CCM 3807) and Yersinia enterocolitica (CCM 5671). Plant extracts were isolated from three plants of Drosera rotundifolia L. (S1, S2 and S3) in different time range. The most effective extract with MIC50 value of 17.07 μg.ml⁻¹ was S3, while forMIC90 of 19.05 μg.ml⁻¹ were extracts S2 and S3 exhibiting antimicrobial activity against Bacillus thuringiensis, Clostridium perfringens and Listeria monocytogenes. Extracts S1, S2 showed MIC50 value 25.53 μg.ml⁻¹ for all the microorganism tested, but S3 extract revealed the same antimicrobial activity against Yersinia enterocolitica, Salmonella enterica subsp. enterica (CCM 3807) and Yersinia enterocolitica (CCM 5671). Plant extracts were isolated from three plants of Drosera rotundifolia L. (S1, S2 and S3) in different time range.
Czech republic). The bacteria were cultured in the nutrient broth for obtaining of bacterial suspension (Imuna, Slovakia) at 37 °C.

### Preparation of plant extracts

Whole plants of *Drosera rotundifolia* L. were dried and crushed. Weights of plant before and after drying are shown in Table 1. Crushed plants were dissolved in 96% ethanol (Sigma, Germany) and stored at room temperature in the dark for two weeks to prevent the degradation of active components. Then, the ethanolic plant extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the ethanol (Stuart RE300DB rotary evaporator, Bibby scientific limited, UK, vacuum pump KNF N838.1.2KT.T5.18, KNF, Germany). For the antimicrobial assay, the crude plant extracts were dissolved in dimethyl sulfoxide (DMSO) (Penta, Czech Republic) to equal 102.4 mg/mL as stock solution, while for chemical analysis ethanol was used as solvent. Analysis of the essential oils was carried out with Hewlett-Packard 5890/5970 GC-MSD system.

### Microbroth dilution method

The minimal inhibitory concentrations (MICs) in vitro of the compounds were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation (CLSI, 2009) in Mueller Hinton broth (Bioline, Italy).

The test samples were dissolved in dimethyl sulfoxide (DMSO) and the stock solutions of the serial two-fold dilutions with the final concentrations ranging between 0.5-512 μg·ml⁻¹ were obtained. After that the each well was inoculated with a 100 μl volume of working microbial suspension at the final density of 0.5 McFarland. Bacterial strains were grown at 37 ± 0.5 °C for 20-24 h. Additionally wells for positive control (wells without bacteria), inoculum viability (no extract added) and the DMSO as negative control were reserved in each plate. The inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with a 100 µl volume of working microbial suspension at the final density of 0.5 ºC for 20 h. Additionally wells for positive control (wells without bacteria), inoculum viability (no extract added) and the DMSO as negative control were reserved in each plate. The inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with a 100 µl volume of working microbial suspension at the final density of 0.5McFarland. Bacterial strains were grown at 37 ± 0.5 °C for 20-24 h. Additionally wells for positive control (wells without bacteria), inoculum viability (no extract added) and the DMSO as negative control were reserved in each plate. The inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with a 100 µl volume of working microbial suspension at the final density of 0.5 ºC for 20-24 h. Additionally wells for positive control (wells without bacteria), inoculum viability (no extract added) and the DMSO as negative control were reserved in each plate. The inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with a 100 µl volume of working microbial suspension at the final density of 0.5 McFarland. Bacterial strains were grown at 37 ± 0.5 °C for 20-24 h. Additionally wells for positive control (wells without bacteria), inoculum viability (no extract added) and the DMSO as negative control were reserved in each plate.

### Statistical analysis

Measurement error was established for 0.05 values of absorbance. Differences in absorbance between the measurements before and after the analysis were expressed as a set of binary values. These values were assigned to exact concentrations. The following formula was created for this specific experiment: value 1 (inhibitory effect) was assigned to absorbance values lower than 0.05, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05. For this statistical evaluation the probit analysis in Statgraphics software was used (Kačániová et al., 2015).

### RESULTS AND DISCUSSION

The *Drosera* genus, native to Australia and New Zealand, includes multiple carnivorous species which possess substantial medicinal potential. Medicinal use of *Drosera* is convenient due to the simplicity of its cultivation in vitro. *Drosera* extracts owe their antimicrobial properties to secondary metabolites. Naphthoquinones, mainly anthraquinones, are the main active compounds produced by *D. binata* tissues. The plants are also a source of flavonoids, ellagic acid and their glycoside and methyl derivatives (Zehl et al., 2011). It is crucial that plant extracts, unlike antibiotics, do not contribute to the emergence of resistant bacterial strains when used as antibacterial agents. Various studies showed interactions between several secondary metabolites found in plant extracts, which allowed herbal drugs to be used in lower doses of active components (Krolicka et al., 2008).

The antimicrobial activity of *Drosera rotundifolia* L. was determined previously by the disc diffusion assay of extracts. Ethanolic extracts of *D. rotundifolia* showed an antimicrobial effect against *Yersinia enterocolitica*, *Bacillus thuringiensis* and *Salmonella enterica* (Kačániová et al., 2014). Carnivorous plant *D. rotundifolia* assumes to be a good source of compounds which possesses antimicrobial effect against different Gram-negative and Gram-positive pathogenic bacteria. In this study the minimal inhibitory concentrations (MICs) of the compounds of individual extracts from three plants of *D. rotundifolia* by the microbroth dilution assay for a quantitative determination were evaluated. Minimum inhibitory concentrations are considered as a great method for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing and MIC is defined as the lowest concentration of a drug, that will inhibit the visible growth of an organisms after incubation (Andrews, 2001).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight before drying</th>
<th>Weight after drying</th>
<th>Sample in DMSO</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>8.82g</td>
<td>0.74g</td>
<td>570 μl</td>
<td>gallic acid 0.5%, hyperoside 0.4%, drosoroside 1%, tanine 0.6%, gallic acid 1.7%, hyperoside 1.2%, drosoroside 2.3%, tanine 1.6%, gallic acid 1.2%, hyperoside 0.6%, drosoroside 1.3%, tanine 0.2%</td>
</tr>
<tr>
<td>S2</td>
<td>11.42g</td>
<td>0.61g</td>
<td>690 μl</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>15.17g</td>
<td>0.82g</td>
<td>480μl</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1** Information about plants extracts

**Figure 1** Antimicrobial activity MIC 50 of *Drosera rotundifolia* L.

**Figure 2** Antimicrobial activity MIC 90 of *Drosera rotundifolia* L extracts (S1, S2 and S3) extracts (S1, S2 and S3)

**BT** - *Bacillus thuringiensis* CCM 19T, **CP** - *Clostridium perfringens* CCM 4991, **LM** - *Listeria monocytogenes* CCM 4609, **EC** - *Escherichia coli* CCM 3986, **SE** - *Salmonella enterica* subsp. enterica CCM 35087, **YE** - *Yersinia enterocolitica* CCM 5671.
The studies on antimicrobial properties of extracts from different species of Drosera are still ongoing and positive antibacterial effect and determination of values MIC was done by Taraszkiewicz et al. (2012), who demonstrated that extracts of Drosera gigantea contain antibacterial compounds that can be used against Pseudomonas syringae. Other author focused on Drosera intermedia extracts and this extract was the most effective against Staphylococcus epidermidis for which a MIC value of 13.0 µg·ml⁻¹ was scored (Grevenstuk et al. 2009). Other Drosera was studied by Didry et al. (1998), who revealed that extract from Drosera peltata showed the broad spectrum activity against numerous bacteria of the oral cavity, with greatest activity against Gram-positive bacteria Strepptococcus mutans and S. sobrinus with MIC value 31.25 µg·ml⁻¹ and 15.63 µg·ml⁻¹, respectively.

Plant extracts are a very rich source of secondary metabolites with antibacterial action, and their application provides an opportunity to effectively combat also antibiotic-resistant bacterial strains (Cushnie & Lamb 2005). Antibacterial activity of species of Drosera demonstrated that extracts obtained from several other in vitro cultured carnivorous plants possess antibacterial activity towards various pathogens in planktonic culture such as Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae and S. aureus. The study of Krychowiak et al. (2014) was the first report describing the efficiency of the D. binata extract itself in eliminating the dangerous human pathogen S. aureus, resulting however in the increased cytotoxicity of extract on human keratinocytes. The antimicrobial effectiveness of the chloroform plant extract was similar towards all studied S. aureus strains, regardless of their resistance to antibiotics. However, a higher bactericidal concentration (MBEC 64 µg·ml⁻¹) was required for in vitro cultured biofilm.

CONCLUSION

In conclusion, the extracts of the tested Drosera rotundifolia exhibited good potential antibacterial activity and the potential for developing of antimicrobial agents. The active extracts should be evaluated further in-depth to isolate other active components and detect their mode of action. Drosera species represent a promising alternative source of material for medicinal use. Drosera extract in this study show very good antimicrobial activity against Gram-positive bacteria.

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REFERENCES


