JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIBACTERIAL ACTIVITY OF *DROSERA ROTUNDIFOLIA* L. AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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doi: 10.15414/jmbfs.2016.5.special1.20-22

ARTICLE INFO

Received 23. 12. 2015
Revised 20. 1. 2016
Accepted 24. 1. 2016
Published 8. 2. 2016

Regular article

OPEN ACCESS

ABSTRACT

The medicinal use of genus *Drosera*, as an important antitussive for different respiratory diseases, has been known for centuries. Many of extracts from carnivorous plants exhibit various antibacterial and antifungal activities. Naphthoquinones containing extracts from *Drosera* have antiviral, antibacterial, antifungal, aphrodisiac, antispasmodic, antileprosy, antisclerotic and anticancer properties. The aim of the present study was to detect antibacterial activity of *Drosera rotundifolia* against Gram-positive and Gram-negative bacteria by the testing of MIC. For the study six strains of microorganisms were selected and there were Gram-positive bacteria -*Bacillus thuringiensis* (CCM 19T), *Clostridium perfringens* (CCM 4991), and *Listeria monocytogenes* (CCM 4699), as well as and Gram-negative bacteria - *Escherichia coli* (CCM 3988), *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 $\mu g.ml^{-1}$ was S3, while forMIC90 of 19.05 $\mu g.ml^{-1}$ were extracts S2 and S3 exhibiting antimicrobial activity against *Bacillus thuringiensis*, *Clostridium perfringens* and *Listeria monocytogenes.* Extracts S1, S2 showed MIC50 value 25.53 $\mu g.ml^{-1}$for all the microorganism tested, but S3 extract revealed the same antimicrobial activity against *Yersinia enterocolitica*, *Salmonella enterica* subsp. *enterica* and *Escherichia coli*. Extract S1 has MIC90 value of 27.14 $\mu g.ml^{-1}$ against all the microorganism tested, but S2 and S3 shared the same MIC90 for *Yersinia enterocolitica*, *Salmonella enterica* subsp. *enterica* and *Escherichia coli.*

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

INTRODUCTION

Plants have several ways of taking up nutrients; among them one of the highly specialized 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 plans 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 platform. They are characterized by growth on the sunny areas, the water-rich sites, but also are able to grow in medium poor of nutrient (**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 (**Šamaj *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 plumbagin,7-methyljuglone and flavonoids (**Banasiuk *et al.*, 2012**). Particularly, naphthoquinones are thought to be responsible for therapeutic effects in respiratory diseases including bronchial infections and tuberculosis. The naphthoquinones, and specially plumbagin, also inhibits a development of parasitic nematodes and insects (**Collantes *et al.*, 2014**). The extracts from *Drosera* which contain naphtoquinones share medical and other valuable properties and the antiviral, antibacterial, antifungal, aphrodisiac, antispasmodic, antileprosy, antisclerotic and anticancer properties are recognized **(Juniper *et al.*, 1989; Šamaj *et al.*, 1999**).
The present study was focused on *Drosera rotundifolia* of genus *Drosera*, which might be a pharmacologically important plant for its antimicrobial activity. The aim of this study was to evaluate the antibacterial activity of *Drosera* plant extracts by the detection of the minimal inhibitory concentration (MIC). Antimicrobial activity of six bacteria: Gram-positive _ *Bacillus thuringiensis*, *Clostridium perfringens*, *Listeria monocytogenes* and Gram-negative bacteria *Yersinia enterocolitica*, *Salmonella enterica* subsp. *enterica* and *Escherichia coli* were evaluated

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 $\mu Em^{-2} s^{-1}$ light intensity. Plant extracts were isolated from three plants of *Drosera rotundifolia* L. in different time range.

Microorganisms

In this study the six bacteria species representing different strains were tested. Among tested were Gram-positive - *Bacillus thuringiensis* CCM 19T, *Clostridium perfringens* CCM 4991, *Listeria monocytogenes* CCM 4699 and Gram-negative bacteria - *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807 and *Yersinia enterocolitica* CCM 5671. All tested strains were collected from the Czech Collection of Microorganisms (Brno,

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 showed 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.45.18, 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.

Table1 Information about plants extracts

Sample	Weight before drying	Weight after drying	Sample in DMSO	Chemical composition
S1	8.82g	0.74g	570 µl	gallic acid 0.5%, hyperoside 0.4%, droseroside 1%, tanine 0.6%
S2	11.42g	0.61g	690 µl	gallic acid 1.7%, hyperoside 1.2%, droseroside 2.3%, tanine 1.6%
S3	15.17g	0.82 g	480µl	gallic acid 1.2%, hyperoside 0.6%, droseroside 1.3%, tanine 0.2%

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 (Biolife, Italy).
The test samples were dissolved in dimethyl sulphoxide (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 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 shaker (Biotek Instruments, USA). The absorbance in 96 microwell plates was measured before and after experiment. Differences between both measurements - prior and after incubation were evaluated as a growth.

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 plumbagin 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 thurigensis* and *Salmonela enterica* (**Kačániová *et al*., 2014**). Carnivorous plant *D. rotundifolia* assumes to be a good source of compounds which posses 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**).

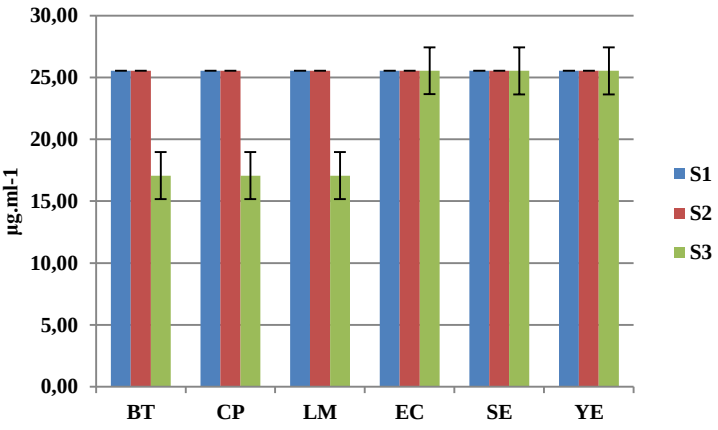

Figure 1 Antimicrobial activity MIC 50 of *Drosera rotundifolia* L.

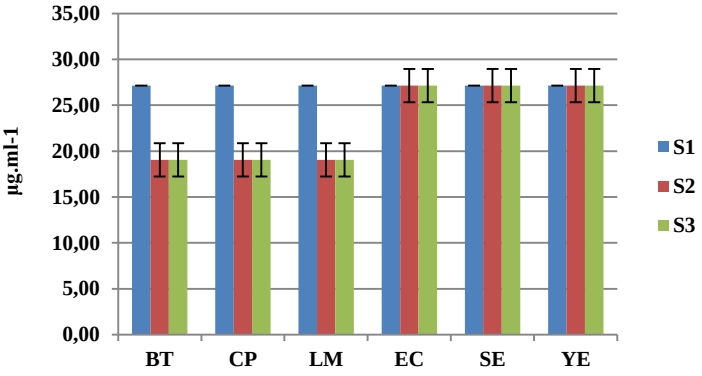

Figure 2 Antimicrobial activity MIC 90 of *Drosera rotundifolia* L extracts (S1, S2 and S3) extracts (S1, S2 and S3)

BT - Bacillus thuringiensis CCM 19T, *CP - Clostridium perfringens* CCM 4991, *LM - Listeria monocytogenes* CCM 4699, *EC - Escherichia coli* CCM 3988, *SE - Salmonella enterica* subsp. *enterica* CCM 3807, *YE - Yersinia enterocolitica* CCM 5671.

The antimicrobial activity (µg.ml^{-1}) of three extracts of *Drosera rotundifolia* L. against various strains of Gram-positive and Gram-negative bacteria are summarized in Figures 1 and 2. The most effective extract with MIC50 value of 17.07 µg.ml^{-1} was extract S3 and with MIC90 of 19.05 µg.ml^{-1} were extracts S2 and S3. The extract S3 with MIC50 was the most active against *Bacillus thuringiensis*, *Clostridium perfringens* and *Listeria monocytogenes*, while the extracts S2 and S3 were the most effective against *Bacillus thuringiensis*, *Clostridium perfringens* and *Listeria monocytogenes* at MIC90. This results correspond with the results of the previous study, where the individual differences between the extracts obtained were observed and extract DR3 showed the best antimicrobial activity against *B. thuringiensis* (**Kačániová *et al*., 2014**). Against *Yersinia enterocolitica*, *Salmonella enteric* subsp. *enterica* and *Escherichia coli* the extracts S1, S2 and S3 showed the same MIC50 value of 25.53 µg.ml^{-1} and MIC90 value of 27.14 µg.ml^{-1}. The extract S1 and S2 have showed the same activity against *Bacillus thuringiensis*, *Clostridium perfringens* and *Listeria monocytogenes* at MIC50. Our results show that Gram-positive bacteria were more susceptible to the addition of plant extract and this could be confirmed with the previous studies where *Bacillus* spp. was susceptible to active compounds of plants extracts **(Didry *et al*., 1998; Krolicka *et al*., 2009)**.

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 $\mu g.ml^{-1}$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 *Streptococcus mutans* and *S. sobrinus* with MIC value 31.25 $\mu g.ml^{-1}$ and 15.63 $\mu g.ml^{-1}$, 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 **(Cuhnie, 2005).**

Previously **Krolicka *et al.* (2008)** 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 $\mu g.ml^{-1}$) 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.

Acknowledgments: This work was supported by grant VEGA 1/0611/14.

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