

Antimicrobial Activity of *Drosera rotundifolia* L.

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Abstract

Droseraceae spp. is widely used in folk medicine. In the present study, the antimicrobial activities of the four *Drosera rotundifolia* L. (D8.11, D15.12, 18.10, 8.11) samples were investigated. The antimicrobial activities were determined by using agar disc diffusion method against grampositive bacteria (*Bacillus thuringiensis*, *Staphylococcus aureus*, *Listeria monocytogenes*) and gramnegative bacteria (*Yersinia enterocolitica*, *Salmonella enteritidis*). The results of the disk diffusion method showed very different activity against all tested strains of microorganisms. The best antimicrobial activity of ethanolic extract *Drosera rotundifolia* L. against *Salmonella enteritidis* was found at *Drosera rotundifolia* (D8.11).

Keywords: antimicrobial activity, agar diffusion method, bacteria, *Drosera rotundifolia* L.

1. Introduction

The use of *D. rotundifolia* in medicinal preparations has been to some extent replaced by imported other species (*Drosera peltata* Sm., *Drosera madagascariensis* DC.) not native to Europe. Although *D. madagascariensis* is poor in active compounds, it has been accepted in pharmacopoeias. The sundews are endangered in many countries and they are protected for example in Belgium. The round-leaved sundew is presently not endangered in Finland, but the small size of plants makes collection from natural stands laborious and therefore, cultivation possibilities have been studied [1].

Drosera species contain physiologically active compounds such as flavonoids, ellagic acid and naphthoquinones [2]. Flavonoids and naphthoquinones have been reported to possess antimicrobial and anti-inflammatory properties, which are efficacious in the treatment of oral infectious diseases. Some anti-inflammatory

agents counteract the effect of neutrophil elastase. The 70 % ethanol extract of *Drosera madagascariensis* and the flavonoids quercetin, hyperoside and isoquercitrin inhibited the human neutrophil elastase [3]. *Droserae* Herba generally comprises dried aerial parts of *Drosera* plants and is usually applied as aqueous or ethanol extract in therapy. Both aqueous and ethanol extracts of *Drosera rotundifolia* inhibited the human neutrophil elastase and induced antispasmodic effect in guinea-pig ileum [4]. Similarly, the extract of *Drosera peltata* and the naphthoquinone, plumbagin, exhibited antimicrobial activity against oral bacteria. Thus the aqueous or ethanol extracts and the constituents of *Drosera* species have been shown to possess the anti-inflammatory properties. In this study, we examined the anti-inflammatory effect of 80 % ethanol extracts of *Drosera* species because the flavonoids and other physiologically active polyphenols are efficiently extracted with 70–80 % ethanol [5].

Consumers life is about changes and development. In some causes, it is question of comeback, in another ones the question of futuristic wishes.

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Nevertheless, the only important thing is to satisfy our customer, but nowadays, do not forget sustainability issues in broaden understanding [6]. The present study was designed to determine the role of ethanolic extracts of *Drosera rotundifolia* L. for potential antibacterial activity against some selected microorganisms as grampositive bacteria: *Bacillus thuringiensis* CCM 19^T, *Staphylococcus aureus* CC 2286, *Listeria monocytogenes* CCM 4699 and gramnegative bacteria *Yersinia enterocolitica* CCM 5671, *Salmonella enterica* subsp. *enterica* CCM 3807.

2. Materials and methods

Plant material

Plants of *Drosera rotundifolia* L. were cultivated *in vitro* on basal MS medium (DUCHEFA) supplemented with 2 % (w/v) sucrose and 0.8 % (w/v) agar [7]. The plantlets were cultivated at 20 ± 20 °C with a day length of 16 h under $50 \mu\text{Em}^{-2} \text{s}^{-1}$ light intensity.

Test microorganisms

Five strains of microorganisms were tested in this research. Grampositive bacteria: *Bacillus thuringiensis* CCM 19^T, *Staphylococcus aureus* CC 2286, *Listeria monocytogenes* CCM 4699 and gramnegative bacteria *Yersinia enterocolitica* CCM 5671, *Salmonella enterica* subsp. *enterica* CCM 3807 were used in this study. 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.

Table 1. Detail information about plants extracts

Sample	Weight before drying	Weight after drying	Sample in DMSO
D8.11.	4.82 g	0.64 g	437 μl
D18.11.	11.42 g	0.51 g	629 μl
D15.11.	5.17 g	0.61 g	4.18 ml
D8.11.	6.97 g	0.70 g	870 μl

Preparation of plant extracts

After drying, *Drosera rotundifolia* L. the materials were crushed, weighed as we showed in tab. 1 and

soaked separately in ethanol p.a. (96 %, Sigma, Germany) during two weeks at room temperature in the dark. Exposure to sunlight was avoided to prevent the degradation of active components. Then, 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, and vacuum pump KNF N838.1.2KT.45.18, KNF, Germany). For the antimicrobial assay, the crude plant extracts were dissolved in dimethyl sulfoxid (DMSO) (Penta, Czech Republic) to equal 102.4 mg/mL as stock solution, while for chemical analysis methanol was used as solvent.

Antibacterial activity with disc diffusion method

Antimicrobial activity of each plant extract and 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.

3. Results and discussion

Nearly 150 species of the carnivorous genus *Drosera* (*Droseraceae*) are distributed mainly in Australia, Africa and South America, with some Northern Hemisphere species [8]. Several Northern Hemisphere species, including *Drosera rotundifolia* L., *Drosera intermedia* Hayne and *Drosera anglica* Huds., have been used as traditional medicine in the therapy of respiratory tract infections. *Droserae* Herba, which is comprised chiefly of *Drosera rotundifolia*, has been commonly used in the treatment of convulsive or whooping cough since the 17th century [9].

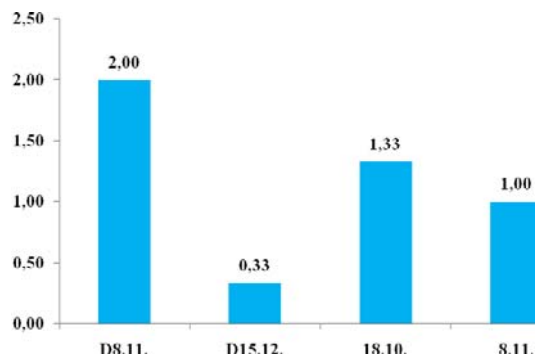


Figure 1 Antimicrobial activity of ethanolic extract of *D. rotundifolia* L. samples against *Y. enterocolitica*

The antimicrobial activity of different samples of *Drosera rotundifolia* L. ethanolic extract against different species of bacteria is showed in figure 1-5.

The best antimicrobial activity against bacteria *Yersinia enterocolitica* was found in sample D8.11. and lowest antimicrobial activity was found in sample D15.12.

The antimicrobial activity of extracts of aerial parts of *Drosera peltata* Smith against oral bacteria was investigated using agar diffusion and dilution micromethods. The chloroformic extract, active against all the bacteria tested, showed the most significant antimicrobial properties. Results obtained suggest that *Drosera peltata* extract could be used in the treatment of oral infectious diseases like dental caries and periodontitis [2].

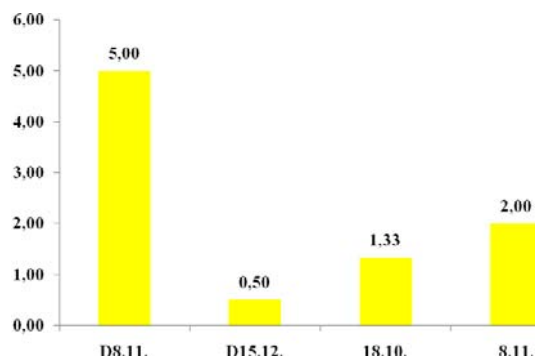


Figure 2 Antimicrobial activity of ethanolic extract of *D. rotundifolia* L. samples against *S. enterica*

The best antimicrobial activity against bacteria *Salmonella enterica* was found in sample D8.11. and lowest antimicrobial activity was found in sample D15.12., what is same as against *Y. enterocolitica*.

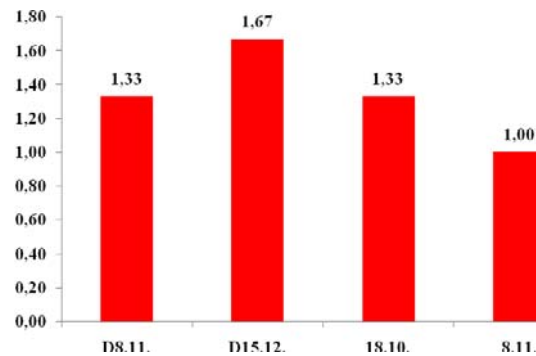


Figure 3 Antimicrobial activity of ethanolic extract of *D. rotundifolia* L. samples against *B. thuringiensis*

The best antimicrobial activity against bacteria *Bacillus thuringiensis* was found in sample D15.12. and lowest antimicrobial activity was found in sample 8.11.

Drosera species contain physiologically active compounds such as flavonoids, ellagic acid and naphthoquinones [2, 11-12]. Flavonoids and naphthoquinones have been reported to possess antimicrobial and anti-inflammatory properties, which are efficacious in the treatment of oral infectious diseases. The 70% ethanol extract of *Drosera madagascariensis* and the flavonoids quercetin, hyperoside and isoquercitrin inhibited the human neutrophil elastase [3]. *Droserae* Herba generally comprises dried aerial parts of *Drosera* plants and is usually applied as aqueous or ethanol extract in therapy. Both aqueous and ethanol extracts of *Drosera rotundifolia* inhibited the human neutrophil elastase and induced antispasmodic effect in guinea-pig ileum [4]. Similarly, the extract of *Drosera peltata* and the naphthoquinone, plumbagin, exhibited antimicrobial activity against oral bacteria [10]. Thus the aqueous or ethanol extracts and the constituents of *Drosera* species have been shown to possess the anti-inflammatory properties. In this study, we examined the anti-inflammatory effect of 80% ethanol extracts of *Drosera* species because the flavonoids and other physiologically active polyphenols are efficiently extracted with 70–80% ethanol.

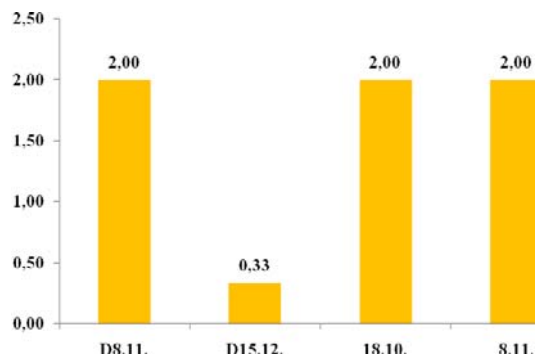


Figure 4 Antimicrobial activity of ethanolic extract of *D. rotundifolia* L. samples against *S. aureus*

The best antimicrobial activity against bacteria *Staphylococcus aureus* was found in samples D8.11. and 18.10 and lowest antimicrobial activity was found in sample D15.12.

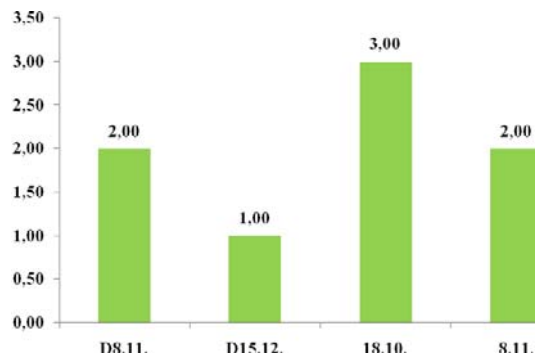


Figure 5 Antimicrobial activity of ethanolic extract of *D. rotundifolia* L. samples against *L. monocytogenes*

The best antimicrobial activity against bacteria *Listeria monocytogenes* was found in samples 18.10. and lowest antimicrobial activity was found in sample D15.12.

4. Conclusions

In conclusion, we can state that the ethanolic extract of *Drosera rotundifolia* L. number D18.11 have the best antimicrobial effect against three bacteria from five. The lowest antimicrobial effect of this ethanolic extract was found in sample number D15.12. In this study was found that this plant material has potential antimicrobial effect against Gram negative and Gram positive pathogenic bacteria.

Acknowledgment

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References

- Kamarainen, T., Uusitalo, Y., Jalonen, J., Laine, K., Hohtol, A., Regional and habitat differences in 7-methyljuglone content of Finnish *Drosera rotundifolia*. *Phytochemistry*, 63, 2013, pp. 309-314.
- Paper, D.H., Karall, E., Kremser, M., Krenn, L., Comparison of the antiinflammatory effects of *Drosera rotundifolia* and *Drosera madagascariensis* in the HET-CAM assay. *Phytotherapy Research*, 2005, 19(4), pp. 323–326.
- Melzig, M.F., Pertz, H.H., Krenn, L., Anti-inflammatory and spasmolytic activity of extracts from *Droserae herba*. *Phytomedicine* 2001, 8 (3), pp. 225–229.
- Krenn, L., Beyer, G., Pertz, H.H., Karall, E., Kremser, M., Galambosi, B., Melzig, M.F., *In vitro* antispasmodic and anti-inflammatory effects of *Drosera rotundifolia*. *Arzneimittelforschung*, 2004, 54(7), pp. 402–405.
- Fukushima, K., Nagai, K., Hoshi, Y., Masumoto, S., Mikami, J., Takahashi, Y., Oike, H., Kobori, M., *Drosera rotundifolia* and *Drosera tokaiensis* suppress the activation of HMC-1 human mast cells. *Journal of Ethnopharmacology*, 2009, 125, pp. 90–96.
- Horská, E., New Consumerism and Trends at the Food Market: within and Beyond Visegrad Borders. In Horská, E. *Food Sciences & Business Studies*. Nitra: SPU, 2012, 410 p. ISBN 978-80-552-0815-2.
- Bobak, M., Blehova, A., Kristin, J., Ovecka, M., Samaj, J., Direct plant regeneration from leaf explants of *Drosera rotundifolia* cultured in vitro. In *Plant Cell Tissue and Organ Culture*, 1995, 43(1), pp. 43-49.
- Rivadavia, F., Kondo, K., Kato, M., Hasebe, M., Phylogeny of the sundews, *Drosera* (Droseraceae) based on chloroplast *rbcL* and nuclear 18S ribosomal DNA sequences. *American Journal of Botany*, 2003, 90, pp. 123–130.
- Didry, N., Dubreuil, L., Trotin, F., Pinkas, M., Antimicrobial activity of aerial parts of *Drosera peltata* Smith on oral bacteria. *Journal of Ethnopharmacology*, 1998, 60 (1), pp. 91–96.
- Budzianowski, J., Naphthohydroquinone glucosides of *Drosera rotundifolia* and *D. intermedia* from *in vitro* cultures. *Phytochemistry*, 1996, 42(4), pp. 1145–1147.
- Krenn, L., Blaeser, U., Hausknot-Chenicek, N., Determination of naphthoquinones in *Droserae herba* by reversed-phase high performance liquid chromatography. *Journal of Liquid Chromatography & Related Technologies*, 1998, 21(20), pp. 3149–3160.