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IN VITRO STUDY OF THE USE OF SOME MEDICINAL PLANTS AGAINST THE FISH PATHOGEN *Aeromonas hydrophila*

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Abstract

In current study the antibacterial activity of different medicinal herbs against fish pathogen Aeromonas hydrophila was evaluated. The water extract of medicinal herbs (Glycyrrhiza glabra, Rhodiola rosea, Althaea officinalis, Sambucus nigra, Inula helenium, Pinus sylvestris, Ocimum basilicum, Salvia officinalis) were prepared by the following method: the plants were extracted in water solution at proportion 1:10. The received homogeny solutions were filtered and centrifuged at 7000 rpm for 30 minutes. Afterwards the extracts were filtered with sterile syringe filters with the size 0.2 μm. The bacterial strain of Aeromonas hydrophila (ATCC 7965) was used in current research. The bacterial activity of plant extract against Aeromonas hydrophila were tested with disk diffusion method. The highest antibacterial effect against Aeromonas hydrophila were determined in water extract of Salvia officinalis and its inhibition zone was higher with 33.34% compared with the size of zone measured for the control variant.

Key words: *Aeromonas hydrophila, antagonistic activity, medicinal plants.*

INTRODUCTION

The aquaculture is one of the fast developing agriculture sectors (Subasinghe et al., 2009). The fast growth in aquaculture production is also associated with the increase of the vulnerability and perceptivity of fish to diseases in aquafarms.

One very important in economics aspect disease which causes high economic losses in aquafarm is hemorrhagic disease. The pathogen associated with this disease is *A. hydrophila* (Maiti et al., 2009; Beaz-Hidalgo et al., 2010). For the treatment of this fish disease usually antibiotics were distributed to fish together with the feed.

Unfortunately it was found that the usage of antibiotics increases the resistance of microorganisms (Sánchez-Romero and Casadesús, 2014). From other side the carcinogen effect of microbiological dyes vastly applied in aquaculture as disinfectants in the past was proved (Srivastava et al., 2004).

These reasons led to necessity alternative therapeutants to be found for the treatment of pathogens in aquaculture. One possible decision for preventing the fish in farms from different diseases is a probiotics (Balcázar et al., 2006; Kesarcodi-Watson et al., 2008). Other possible variant for the counteraction to diseases in aquaculture are essential fatty acids (Randrianarivelo et al., 2010).

Different extracts from medicinal plants were successfully used for the treatment of different pathogens in aquaculture in the last decade (Bansemir et al., 2006; Turker et al., 2009).

Bulgaria is reach of medicinal plants which are used from Bulgarian traditional medicine for treatment of human diseases from hundreds of years. These medicinal plants could possibly be used for the treatment of different fish pathogens including *Aeromonas hydrophila*.

The inhibition effect of extracts of different medicinal plants against fish pathogen *Aeromonas hydrophila* was tested *in vitro* in current study.

MATERIALS AND METHODS

Preparation of water extracts from different medicinal plants

Water extract of medicinal plants were made according to Dellavalle et al. (2011). The plants were bought from herbal pharmacy and were extracted in water solution at proportion 1:10. The received homogeny solutions were filtered

and centrifuged at 7000 rpm for 30 minutes. Afterwards the extracts were filtered with sterile syringe filters with the size 0.2 µm (Minisart, Sartorius Stedim Biotech GmbH, Germany). The medical plants and the morphological parts which were used in current experiments were shown in Table 1.

Table 1. The morphological parts of medicinal plants used in experiments

Medicinal plants	Morphological parts of medicinal herbs
Golden root (*Rhodiola rosea*)	Leaf, stem and blossom
Mashmallow (*Althaea officinalis*)	Root
Elderberry (*Sambucus nigra*)	Blossom
Elecampane (*Inula helenium*)	Root
Scots pine (*Pinus sylvestris*)	Tips
Basil (Ocimum basilicum)	Leaf and stem
Sage (Salvia officinalis)	Leaf and stem

The strain of fish pathogen *Aeromonas hydrophila* ATCC 7965 was used in tests.

In vitro* tests of extracts from medicinal plants against fish pathogen *Aeromonas hydrophila

The suspension of bacteria *Aeromonas hydrophila* with concentration $1.2x10^6$ CFU/ml was spread on Petri dishes contained Mueller-Hinton agar. Disc diffusion method was used for determination of inhibition of fish pathogen *Aeromonas hydrophila* from extracts of different medicinal plants. Prior *in vitro* tests sterile discs (Himedia) with diameter 6 mm were impregnated with different plant extracts and were placed with sterile pincers on sterile media. The Petri dishes were incubated for 48 hours at 28°C. Antibacterial activity of extracts was determinate by measurement the diameter of inhibition zones. The disc impregnated with distilled water was used as a control variant.

Data analysis

The statistical differences in received data were checked by ANOVA (MS Office, 2010).

RESULTS AND DISCUSSIONS

Furmanowa et al. (2002), found antimicrobial effect of *Rhodiola rosea* roots and callus extract on some strain of *Staphylococcus aureus*. *In vitro* tests with water extract of golden root, against fish did not show inhibition effect against fish pathogen *A. hydrophila* and the diameter of its inhibition zone was 6 mm (Figure 1).

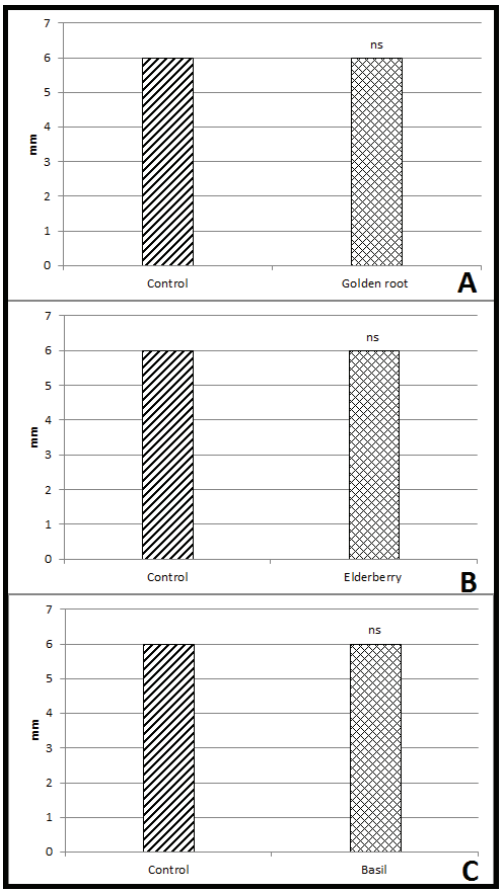

Figure 1. Diameter of inhibition zone in control and experimental variants: extract from golden root (*Rhodiola rosea)* - A; extract from elderberry (*Sambucus nigra*) – B; extract from basil (*Ocimum basilicum*) - C

Cioch et al. (2017) stated that aqueous extracts of elderberry showed antimicrobial activity in higher concentration (> 4 mg/ml). Tested from us concentration of elderberry did not show inhibition effect against fish pathogen *A. hydrophila* (Figure 1).

In vitro tests with water extract of basil (*Ocimum basilicum*) against *A. hydrophila* did not show inhibition effect against this fish pathogen, and the diameter of its inhibition zone was 6 mm. Runyoro et al. (2010) found

that *Ocimum basilicum* oil showed weak antibacterial activity.

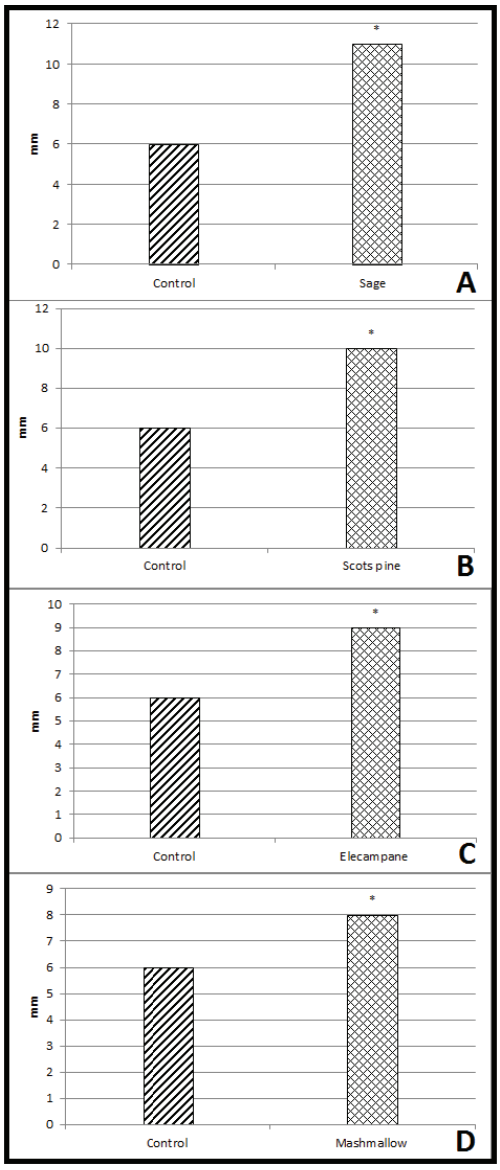

Figure 2. Diameter of inhibition zone in control and experimental variants: extract from sage (*Salvia officinalis*) - A; extract from scots pine (*Pinus sylvestris*) - B; extract from elecampane (*Inula helenium*) - C; extract from mashmallow (*Althaea officinalis*) - D

The aqueous extracts of other four tested medicinal plants showed inhibition effect against *A. hydrophila*. The inhibition effect of sage against fish pathogen was the highest, followed by this found in the extract from *Pinus sylvestris* tips and the weakly inhibition effect was found for the extract from elecampane and mashmallow (Figure 2). The inhibition zone of sage was higher with 33.34% compared with the diameter of zone measured for the control variant. Our results are in line with the results found from Delamare et al. (2007), who stated that the oil of sage possess remarkable bacteriostatic and bactericide activity against different pathogens - *Bacillus cereus*, *Bacillus*, *Aeromonas hydrophila*, *Aeromonas sobria,* and *Klebsiella oxytoca*. Maruzzella and Lichtenstein (1956) found that the oil from scots pine had bactericide activity against different Gram-positive and Gram-negative bacteria.

CONCLUSIONS

The inhibition effect of sage against fish pathogen *Aeromonas hydrophila* was the highest, followed by this found in the extract from *Pinus sylvestris* tips and the weakly inhibition effect was found for the extract from elecampane and mashmallow. *In vitro* tests conducted from us did not find inhibition effect of extracts from golden root, elderberry and basil against fish pathogen *Aeromonas hydrophila* ATCC 7965.

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