



SELECTION OF SUBSTANCES FOR CONTROLLING AND TREATING MOTILE AEROMONAS HEMORRHAGIC SEPTICEMIA IN DISCUS FISH (*SIMPHYSODON SPP.*)

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RESUMO

Bacterial diseases are one of the main threats during the ornamental fish trade. Discus fish (*Simphysodon* spp.) is one of the most exported fish in the Amazon, and antimicrobials are limited in Brazil, which raises concerns within the context of One Health. Alternative antimicrobials with the potential to treat bacterioses are urgent and needed. This study aimed to select compounds with antimicrobial activity against *Aeromonas hydrophila* based on *in vitro* tests. The antibiogram-assay followed the Kirby-Bauer protocol in agar Muller-Hinton. The susceptibility of the target-strain against the antimicrobials was classified as resistant (diameter ≤ 14 mm), intermediate ($15 \text{mm} \leq \text{diameter} \leq 19$ mm), and susceptible (≥ 20 mm) following the Clinical Laboratory Standard Institute (CLSI). Eight commercial antimicrobials were investigated (triplicate) against *A. hydrophila*: amoxicillin (10mg), erythromycin (15mg), neomycin (30mg), florfenicol (30mg), norfloxacin (10mg), amoxicillin with clavulanate (10mg), sulfazotrim (25mg) and oxytetracycline (30mg). For the microdilution assay, individual minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined following CLSI for 16 herbal medicines (essential oils), two isolated compounds (thymol and carvacrol), and two antibiotics (oxytetracycline and florfenicol). The compounds were classified through their mode of action as bactericidal or bacteriostatic based on the MBC/MIC ratio. Results between 1-4 were classified as bactericidal and ≥ 8 as bacteriostatic. The antimicrobial activity of the target compounds (except antibiotics) was classified based on the MIC result as strong ($\text{MIC} \leq 0.5 \text{mg/mL}$); moderate ($0.6 \leq \text{MIC} \leq 1.5 \text{mg/mL}$); and weak ($\text{MIC} \geq 1.6 \text{mg/mL}$). Florfenicol was classified as susceptible, intermediate, or resistant if $\text{MIC} \leq 4$, 8, and >8 , respectively, while oxytetracycline was ≤ 1 , 2, and >2 , respectively. *A. hydrophila* showed resistance to 5 antimicrobials (62.5% of antimicrobials tested) and susceptibility to only three: florfenicol, norfloxacin, and sulphazotrim. Regarding the essential oils, 11 were classified as bactericidal (68.75%), and the antimicrobial activity ranged between weak (27.2%), moderate (36.4%), and strong (36.4%). The essential oils that show strong activity were: *Syzygium aromaticum* (clove), *Thymus vulgaris* (thyme), *Cymbopogon* sp. (citronella), *Cinnamomum verum* (cinnamon). Both isolated compounds were bactericidal and presented moderate antimicrobial activity. Florfenicol was bacteriostatic and susceptible to *A. hydrophila*, while oxytetracycline was bacteriostatic and resistant against the same target strain. This study highlights four essential oils with strong activity against *A. hydrophila* from discus fish and emphasizes the

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importance of investing in sanitary protocols with these natural compounds, with the potential to diminish the environmental impact and mitigate antimicrobial resistance, preserving the health of aquatic and human ecosystems.

PALAVRAS-CHAVE: *Aeromonas hydrophila*, Antibiotic, Herbal medicine, Ornamental fish

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