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Phytochemical Characterization and Anaesthetic Efficacy of Citrus Leaf Extracts for Sedation and Handling of Nile tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*)

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Abstract This study investigated the anesthetic efficacy and safety of aqueous leaf extracts of *Citrus sinensis*, *Citrus aurantium*, and *Citrus limon* in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) under controlled immersion conditions. Qualitative phytochemical screening revealed distinct variation in bioactive constituents among the extracts. Experimental exposure was conducted at concentrations ranging from 1 000 to 4 000 mg L⁻¹, and responses were evaluated using induction time, recovery time, survival, behavioural indicators, and flesh quality parameters. Anesthetic effects were concentration dependent in both species. *Citrus sinensis* produced mild to moderate sedation across all tested concentrations, with no mortality recorded even at 4 000 mg L⁻¹, indicating a wide safety margin, and 3 000 mg L⁻¹ was identified as the highest effective concentration for routine handling. In contrast, *Citrus aurantium* and *Citrus limon* induced deeper anesthetic states at lower concentrations but resulted in 100 percent mortality at 4000 mg L⁻¹ in both species. Fish exposed to *Citrus sinensis* exhibited more favourable post exposure welfare indicators, including faster recovery and earlier resumption of feeding, whereas the other extracts were associated with delayed recovery and behavioural impairment. These findings indicate that *Citrus sinensis* appears more compatible with short term handling welfare and represents a practical and cost effective botanical anesthetic for freshwater aquaculture.

Keywords Fish anesthesia; Citrus leaf extracts; *Clarias gariepinus*; *Oreochromis niloticus*; Handling stress; Aquaculture welfare

1 Introduction

Aquaculture has become an essential component of global food systems, contributing significantly to animal protein supply and economic development, particularly in developing regions. Species such as *Clarias gariepinus* and *Oreochromis niloticus* are widely cultivated due to their adaptability, rapid growth, and high market acceptance (Klimuk et al., 2024; Webster and Lim, 2024). However, routine aquaculture practices such as handling, grading, transport, and sampling expose fish to stress, which can negatively affect physiological stability, immune function, and overall productivity (Martos Sitcha et al., 2020; Dawood et al., 2022). The use of anesthetic agents is therefore essential to reduce stress, improve handling efficiency, and enhance fish welfare during aquaculture operations (Neiffer, 2021; Brønstad, 2022).

Conventional fish anesthetics, including synthetic compounds, have been widely used due to their effectiveness in inducing rapid sedation and recovery. However, concerns have been raised regarding their cost, regulatory restrictions, potential toxicity, and residue accumulation in fish tissues (Vergneau Grosset and Benedetti, 2022; Sedyaaw and Bhatkar, 2024). These limitations have prompted increasing interest in the development of alternative anesthetic agents derived from natural sources. In particular, plant based anesthetics have gained attention due to their accessibility, lower environmental impact, and perceived safety in aquaculture systems (Yaşar and Yardımcı, 2022; Haihambo et al., 2023).