Recent studies have demonstrated that plant derived compounds, especially those obtained from essential oils, can effectively induce anesthesia in fish. For example, eugenol based extracts and other bioactive plant oils have been shown to produce rapid induction and acceptable recovery profiles in several aquaculture species (Ventura et al., 2020; Zahran et al., 2021). Similarly, essential oil extracts such as chamomile oil and citronellal have been reported to exhibit anesthetic efficacy in fish, influencing behavioural and physiological responses during exposure (Ak et al., 2022; Hoseini et al., 2022). Reviews have further highlighted the growing application of essential oils as sedatives and anesthetics in aquaculture, with evidence supporting their role in improving fish handling and reducing stress (Rodrigues Brandão et al., 2022; Minaz et al., 2025). These findings indicate that plant based anesthetics represent a viable alternative to conventional synthetic agents.

Despite these advances, several limitations remain in current knowledge. Most studies have focused on a limited number of plant species, particularly those rich in essential oils, while comparatively less attention has been given to aqueous leaf extracts from widely available tropical plants. In addition, there is limited comparative research evaluating multiple plant species under similar experimental conditions, especially with respect to induction time, recovery dynamics, survival outcomes, and post exposure welfare indicators (Haihambo et al., 2023; Mphande et al., 2023). Furthermore, the relationship between phytochemical composition and anesthetic performance is not consistently established, as many studies do not integrate chemical profiling with functional assessment of anesthetic effects.

Citrus species represent a promising but underexplored source of bioactive compounds with potential anesthetic properties. Citrus leaves and by products are known to contain a wide range of phytochemicals, including flavonoids, limonoids, terpenoids, carotenoids, and phenolic compounds, many of which exhibit biological activity (Addi et al., 2021; Saini et al., 2022; Lu et al., 2023). Flavonoids and related compounds have been associated with antioxidant, antimicrobial, and physiological regulatory effects, which may influence stress response and metabolic processes in aquatic organisms (Barreca et al., 2020; Bhowal et al., 2022). In addition, citrus leaf extracts and essential oils have demonstrated bioactive properties, including antimicrobial and antiproliferative activities, indicating their potential for broader biological applications (Asker et al., 2020; Othman et al., 2022). The availability of citrus waste and leaf biomass further enhances their relevance as cost effective and sustainable resources for aquaculture applications (Russo et al., 2021; Maqbool et al., 2023; Šafranko et al., 2023).

However, despite the documented phytochemical richness of citrus species, their anesthetic potential in fish has not been systematically evaluated. Existing studies have largely focused on nutritional, antimicrobial, or pharmaceutical properties, with limited attention to their functional role as anesthetic agents in aquaculture systems (Leporini et al., 2020; Zahr et al., 2023). Moreover, comparative assessments of different citrus species under controlled experimental conditions remain scarce, particularly in relation to key performance indicators such as induction efficiency, recovery time, survival rate, and post exposure behavioural responses.

In this context, the present study aims to address these gaps by evaluating the anesthetic efficacy of aqueous leaf extracts of *Citrus sinensis*, *Citrus aurantium*, and *Citrus limon* in Clarias gariepinus and *Oreochromis niloticus*. Specifically, the study integrates phytochemical screening with functional assessment of induction time, recovery patterns, mortality outcomes, and welfare related behavioural responses. By providing a comparative analysis across multiple citrus species and linking phytochemical composition to anesthetic performance, this study contributes new evidence toward the development of plant based anesthetic alternatives for sustainable aquaculture practices. This study represents one of the first comparative evaluations of aqueous citrus leaf extracts as anesthetic agents in tropical aquaculture species.

2 Materials and Methods

2.1 Study location and experimental fish

The experiment was conducted at the aquaculture research facilities of the Department of Fisheries and Aquaculture, Delta State University, Abraka, Nigeria. Nile tilapia (*Oreochromis niloticus*) and African catfish