(*Clarias gariepinus*) were selected because they dominate aquaculture production in sub Saharan Africa and exhibit distinct physiological and behavioural responses to handling stress, making them appropriate models for anesthetic evaluation (Musa et al., 2021; Klimuk et al., 2024).

A total of 180 healthy adult fish comprising 90 Nile tilapia and 90 African catfish were obtained from a commercial aquaculture facility in Warri, Delta State. The fish were size matched to ensure experimental consistency, with Nile tilapia having a mean body weight of 130 ± 10 g and total length of 16 ± 2 cm, while African catfish had a mean body weight of 200 ± 20 g and total length of 22 ± 3 cm. Fish were transported in aerated containers and acclimated for three weeks in 1 000 L circular tanks under continuous aeration. Stocking density was regulated to minimise crowding stress, and fish were fed once daily with a commercial extruded diet. Water quality parameters were monitored throughout acclimation and maintained within recommended ranges for tropical freshwater species to ensure that observed responses were attributable to treatment effects rather than environmental variation (Shaw et al., 2022; Zidan et al., 2022).

Treatments that resulted in complete mortality were excluded from inferential statistical analysis because their inclusion would have introduced perfect separation of outcomes and artificially inflated variance, thereby violating the assumptions of parametric testing. Under such conditions, descriptive reporting is considered more appropriate and is widely adopted in fish anesthesia research where lethal thresholds produce non-variable outcomes (Neiffer, 2021; Soldatov, 2021)

2.2 Plant material collection and extract preparation

Fresh leaves of *Citrus sinensis*, *Citrus aurantium*, and *Citrus limon* were collected from the university botanical garden, washed with distilled water, and air dried under shade at ambient temperature to preserve heat sensitive phytochemicals, as recommended for maintaining the integrity of plant secondary metabolites (Asker et al., 2020; Leporini et al., 2020).

For phytochemical screening, dried leaves were milled into powder. Thirty grams of each sample were macerated in 120 mL of solvent for 12 h at 25 °C, followed by filtration, concentration using rotary evaporation, and drying in a water bath. The extraction procedure yielded approximately 8 to 12 percent of dry extract relative to initial plant mass, which is consistent with reported recovery ranges for citrus leaf phytochemicals (Cebadera Miranda et al., 2020). The dried extracts were reconstituted to 1 mg mL^{-1} for qualitative analysis (Cebadera Miranda et al., 2020; Othman et al., 2022).

For anesthetic trials, fresh leaves were homogenized in sterile distilled water and filtered through muslin cloth to obtain crude aqueous extracts. Filtration effectively removed coarse particulate material, although fine suspended particles remained, reflecting the use of minimally processed extracts. The reported concentrations therefore represent the mass of fresh plant material per unit volume of water rather than purified extract mass. Phytochemical screening was conducted using dried extracts to provide general chemical characterization, whereas fresh aqueous homogenates were used in exposure trials to simulate preparation methods applicable under practical aquaculture conditions. This dual approach ensured alignment between laboratory based analysis and field relevant application (Indriyani et al., 2023; Maqbool et al., 2023).

2.3 Phytochemical screening

Qualitative screening was conducted to detect flavonoids, limonoids, terpenoids, phenolic acids, carotenoids, coumarins, essential oils, and alkaloids. These compound classes were selected based on documented associations with sedative activity, antioxidant function, and modulation of physiological responses in fish (Barreca et al., 2020; Bhowal et al., 2022; Šafranko et al., 2023).

2.4 Experimental design and anesthetic exposure

Fish of each species were randomly assigned to four extract concentrations of 1 000, 2 000, 3 000, and 4 000 mg L^{-1}. Each treatment was replicated three times with ten fish per replicate, resulting in thirty fish per treatment per species.