2.6 Flesh quality assessment

Flesh quality evaluation was undertaken on fish exposed to citrus leaf extracts at a concentration of 3 000 mg L^{-1}, which elicited clear anesthetic responses without causing immediate mortality in the treatments considered. After complete behavioural recovery, fish were humanely euthanised in line with established practices for aquaculture research. Dorsal muscle tissues were excised immediately after euthanasia, placed on ice, and analysed within six hours in order to minimise post mortem biochemical changes that could influence flesh quality parameters (Shadieva et al., 2020; Ventura et al., 2020).

Muscle pH was determined using a calibrated digital pH meter inserted into homogenised muscle tissue, a method routinely employed to assess post exposure metabolic condition and flesh stability in cultured fish species (Shadieva et al., 2020; Zahran et al., 2021). Crude protein content was analysed using standard wet chemistry procedures widely applied in fish nutrition and flesh composition research, while lipid content was quantified through solvent extraction techniques appropriate for detecting variations in muscle lipid reserves associated with handling stress and anesthetic exposure (Fawole et al., 2020; Shadieva et al., 2020). All biochemical determinations were conducted in triplicate, and results were expressed on a wet weight basis to ensure consistency with established reporting practices in aquaculture studies.

Organoleptic assessment was performed to examine potential post anesthetic effects on flesh characteristics relevant to consumer acceptance. Evaluated attributes included flesh odour, texture, colour, and the presence or absence of off flavour characteristics. Sensory evaluation was conducted by a trained panel using established descriptive criteria commonly adopted in studies assessing the influence of handling stress and anesthetic agents on fish flesh quality (Ventura et al., 2020; Russo et al., 2021). These evaluations were qualitative in nature and intended to identify pronounced alterations in sensory attributes rather than to provide detailed quantitative sensory profiling, in line with the applied objectives of fisheries and aquaculture research (Zahran et al., 2021).

2.7 Ethical consideration

All experimental procedures involving fish were carried out in compliance with internationally recognised guidelines governing the care and use of aquatic animals in research. Handling time and exposure duration were kept to the minimum necessary to limit stress, and any fish showing signs of severe distress were promptly removed from the experimental tanks. Throughout the study, established institutional best practices for ethical research involving live aquatic organisms were strictly observed.

3 Results

All tables and figures are explicitly referenced within the text. Phytochemical variation among the citrus extracts is presented (Table 1), behavioural responses are summarised (Table 2 and Tables 3), mortality outcomes are shown (Table 4), and recovery dynamics are quantitatively illustrated (Figure 3), while qualitative behavioural recovery patterns are shown (Figure 1)" (Neiffer, 2021; Mphande et al., 2023).Statistical analyses were conducted using tank means, with the tank treated as the experimental unit (n = 3 per treatment). Although this level of replication is consistent with controlled aquaculture experiments, the relatively small sample size may limit statistical power and should be considered when interpreting the results.

3.1 Phytochemical composition of citrus leaf extracts

The qualitative phytochemical composition of the aqueous citrus leaf extracts is presented (Table 1). Distinct variation was observed in the distribution of bioactive compounds among the three citrus species.

Citrus sinensis contained flavonoids, limonoids, terpenoids, phenolic acids, carotenoids, and coumarins, but did not show detectable levels of essential oils or alkaloids. *Citrus aurantium* contained flavonoids, limonoids, carotenoids, coumarins, essential oils, and alkaloids, but lacked terpenoids and phenolic acids. In contrast, *Citrus limon* contained limonoids, phenolic acids, coumarins, and essential oils, but lacked flavonoids, terpenoids, carotenoids, and alkaloids. These findings are based on qualitative phytochemical screening and were not