with larger sample sizes and more robust experimental designs are required to confirm these results and enhance their general applicability.

Although regression based dose response modelling can provide additional quantitative insight, the primary objective of this study was to identify practical anesthetic thresholds, recovery dynamics, and safety margins under discrete treatment conditions. Accordingly, concentration levels were selected based on established protocols in botanical anesthetic research, which commonly employ stepwise exposure ranges to distinguish sedation, anesthesia, and toxicity thresholds rather than continuous modelling approaches (Ventura et al., 2020; Hoseini et al., 2022). Figure 1 and Figure 3 present complementary but conceptually distinct aspects of anaesthetic response. Figure 1 illustrates qualitative recovery patterns, capturing behavioural restoration such as equilibrium, swimming coordination, and post exposure activity, which are widely recognised indicators of physiological recovery and welfare status in fish (Martos Sitcha et al., 2020; Mphande et al., 2023). In contrast, Figure 3 provides quantitative measurements of induction and recovery time, reflecting the temporal dynamics of anaesthetic uptake and elimination (Neiffer, 2021). While Figure 1 emphasises the quality of recovery, Figure 3 defines the rate and duration of anaesthetic processes, thereby offering complementary evidence for evaluating efficacy and safety (Vergneau Grosset and Benedetti, 2022).

4 Discussion

4.1 Phytochemical basis of anaesthetic activity

Figure 1 represents qualitative behavioural recovery, whereas Figure 3 quantifies induction and recovery time dynamics, ensuring clear separation of functional interpretation (Neiffer, 2021; Mphande et al., 2023).

The variation in anaesthetic performance observed across the citrus leaf extracts can be more meaningfully interpreted in relation to their phytochemical composition as presented (Table 1), rather than as isolated behavioural outcomes. Citrus species are well established sources of diverse bioactive compounds, including flavonoids, phenolic acids, terpenes, and essential oils, many of which exert measurable physiological effects in aquatic organisms (Saini et al., 2022; Lu et al., 2023). The patterns observed in Tables 2 to 6 indicate that anaesthetic activity is not determined by a single compound class but rather by the interaction between neuroactive constituents and compounds that maintain cellular stability.

Extracts characterised by the presence of flavonoids and phenolic acids, as shown for *Citrus sinensis* (Table 1), were associated with gradual behavioural transitions and consistent recovery timing illustrated (Figure 3, Table 5), with behavioural recovery quality clarified in Figure 1" (Neiffer, 2021; Mphande et al., 2023). This observation is consistent with established evidence that citrus flavonoids function primarily as antioxidants that stabilise cellular membranes and reduce oxidative stress rather than directly inducing central nervous system depression (Addi et al., 2021; Barreca et al., 2020). Such compounds have been shown to support physiological resilience under stress conditions, thereby facilitating reversible sedation rather than deep anaesthesia (Šafranko et al., 2023). This mechanistic role explains the relatively controlled anaesthetic responses and wide safety margin observed in Table 6.

In contrast, extracts containing essential oils, particularly *Citrus aurantium* and *Citrus limon* as indicated in Table 1, produced more rapid and pronounced behavioural depression, as reflected in Table 2 and Table 3, and were associated with increased mortality at higher concentrations as shown in Table 4 and Figure 2. Essential oils are known to contain volatile terpenoid compounds that interact directly with neural pathways and induce central nervous system depression in fish (Rodrigues Brandão et al., 2022). However, these compounds have also been shown to impair gill function and reduce oxygen uptake during immersion exposure, particularly at elevated concentrations (Soldatov, 2021). This dual effect provides a clear explanation for the high potency but reduced safety margin associated with these extracts.

The presence of alkaloids in *Citrus aurantium* (Table 1) further contributes to this interpretation. Alkaloids are recognised for their capacity to alter neurotransmission and ion channel activity, thereby enhancing sedative effects but also increasing the risk of toxicity under higher exposure levels (Bhowal et al., 2022). The combined