Larvae were fed standardized diets such as finely ground fish food or yeast suspensions to promote consistent growth rates, while adult mosquitoes were provided with sugar solutions for sustenance and periodic blood meals when necessary for egg production. Rearing containers were regularly cleaned to prevent microbial contamination that could affect mosquito health or experimental outcomes. These controlled rearing protocols ensured that test mosquitoes exhibited normal development and behavior suitable for reliable bioassay results (Priya and Jones, 2021; Dutta and Dey, 2023).

2.3 Bioassay methods and experimental design

Bioassays followed standardized World Health Organization (WHO) protocols adapted for evaluating larvicidal and adulticidal effects of plant extracts against *Anopheles gambiae*. Larval bioassays involved exposing early fourth instar larvae to various concentrations of plant extracts diluted in water, with mortality recorded after 24 hours of exposure. Control groups received solvent only to account for any non-specific effects. Concentration-mortality data were analyzed using probit analysis to determine lethal concentration values (LC50 and LC90), indicating the potency of each extract (Ravi et al., 2018; Pavela et al., 2019).

Adulticidal assays entailed topical application or exposure of adult mosquitoes to treated surfaces impregnated with plant extracts, monitoring knockdown rates and mortality over specified time intervals. Experimental designs incorporated replicates per treatment concentration alongside controls to ensure statistical robustness. Additionally, sub-lethal effects such as behavioral changes or reproductive impairments were observed where applicable. This comprehensive approach allowed assessment not only of acute toxicity but also potential impacts on mosquito population dynamics relevant for vector control strategies (Hafsi et al., 2022; Dutta and Dey, 2023).

3 Chemical Composition Analysis of Plant Extracts

3.1 Determination of physicochemical properties of extracts

The physicochemical properties of plant extracts are fundamental for understanding their stability, solubility, and bioactivity, which influence their efficacy as mosquito control agents. Parameters such as pH, viscosity, density, and refractive index are commonly measured to characterize the extracts and ensure consistency across batches. These properties can affect the interaction of bioactive compounds with mosquito targets and their formulation into usable products. For example, pH can influence the ionization state of active molecules, altering their penetration or binding affinity (Godlewska et al., 2023; Zhang et al., 2023).

Additionally, preliminary qualitative tests such as colorimetric assays help identify classes of phytochemicals present in the extracts, including phenols, flavonoids, alkaloids, tannins, and saponins. These compounds often contribute to insecticidal activity through various mechanisms like enzyme inhibition or membrane disruption. Rapid screening methods provide an initial profile that guides more detailed chemical analyses and bioassays. Maintaining standardized physicochemical characteristics is essential for reproducibility in experimental evaluations and potential field applications (Heinrich et al., 2022; Godlewska et al., 2023).

3.2 Identification of active components

Gas chromatography-mass spectrometry (GC-MS) is a widely used analytical technique for identifying volatile and semi-volatile compounds in plant extracts. It separates complex mixtures into individual constituents based on their retention times and mass spectra, allowing precise identification by comparison with spectral libraries. GC-MS analysis typically reveals a diverse array of bioactive molecules such as terpenes, hydrocarbons, alcohols, esters, and fatty acids that may contribute to larvicidal or adulticidal effects against *Anopheles gambiae* (Khdera and Saad, 2024; Dhanaraj et al., 2025).

High-performance liquid chromatography (HPLC) complements GC-MS by enabling the separation and quantification of non-volatile or thermally labile compounds like polyphenols, flavonoids, and alkaloids. Coupled with detectors such as UV-Vis or mass spectrometry (LC-MS), HPLC provides detailed profiles of major phytochemicals responsible for biological activity. Combining these chromatographic techniques ensures comprehensive characterization of plant extracts' chemical composition critical for correlating specific compounds with observed insecticidal properties (Figure 2) (Heinrich et al., 2022; Hodoșan et al., 2025).