2 Materials and Methods

2.1 Source of plant materials and extraction procedures

Plant materials used for this study were collected from local sources, ensuring freshness and proper identification before extraction. The extraction process involved selecting appropriate solvents based on the polarity of target bioactive compounds, commonly including polar solvents such as methanol or ethanol, which are effective in extracting a wide range of phytochemicals. Extraction methods such as maceration and Soxhlet extraction were employed to maximize yield; maceration involves soaking plant material in solvent at room temperature for extended periods, while Soxhlet extraction uses continuous solvent reflux to enhance compound recovery. These methods have been widely used in mosquito control research due to their efficiency in isolating larvicidal and adulticidal compounds from plants (Figure 1) (Ravi et al., 2018; Abubakar and Haque, 2020).

Figure 1 Morphological midgut content induced by *Azolla pinnata* plant extract from soxhlet extraction method in larvae of *Ae. Aegypti* (Adopted from Ravi et al., 2018)

Image caption: (A) Control test for midgut content view in early 4th instar larvae of *Ae. Aegypti* (B) A. pinnata crude extract for midgut content view in larvae of *Ae. Aegypti*. Arrows indicating the plant extracts (greenish colour), GC: gut content (after 24hours) (Adopted from Ravi et al., 2018)

Following extraction, crude extracts were concentrated under reduced pressure using rotary evaporation to remove solvents without degrading active constituents. The chemical composition of extracts was characterized using chromatographic techniques like gas chromatography-mass spectrometry (GC-MS) to identify major phytochemicals responsible for insecticidal activity. Such characterization is essential for understanding the bioactive profile and guiding further bioassays. Quality control measures included standardizing extract concentrations and storing samples at low temperatures to preserve stability prior to testing (Abutaha and Al-Mekhlafi, 2020; Hafsi et al., 2022).

2.2 Origin of experimental mosquitoes and laboratory rearing conditions

Experimental *Anopheles gambiae* mosquitoes were obtained from established laboratory colonies maintained under controlled environmental conditions to ensure uniformity in age, physiological status, and genetic background. Colonies were typically reared at temperatures between 25-28°C with relative humidity around 70-80%, simulating natural tropical environments conducive for mosquito development. Photoperiods were maintained on a 12:12 hour light-dark cycle to regulate circadian rhythms affecting mosquito behavior and physiology (Ahamd et al., 2023; Dutta and Dey, 2023).