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In Vitro Toxicity Levels of *Urtica massaica* Mildbr (Family: *Urticaceae*) on *Anopheles gambiae* Giles (Diptera: Culicidae) Mosquitoes

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Abstract Botanicals are targets for green insecticides and alternatives to synthetic insecticides. In this study, a randomized experimental design with control was used to evaluate *in vitro* toxicity level (LC_{50} and LC_{90}) of crude methanol and hexane *Urtica massaica* leaf, stem and root extracts on immature stages of *Anopheles gambiae*. 100 eggs, larvae or pupae were exposed to doses of 80 mg/100mls (e/w), 40 mg/100mls (e/w), 20 mg/100mls (e/w), 10 mg/100mls (e/w), 5 mg/100mls (e/w), 2.5 mg/100mls (e/w) of the extracts in clear plastic containers measuring 6 cm × 5.7 cm × 3.5 cm. Each container held 33 mls of a dose and either 33 eggs, larvae or pupae. The experiments were replicated four times. The set ups were left to stand overnight except that of eggs that stood for 48hrs. Mortality was assessed at the end of the period. It was found that methanol extracts were more toxic than hexane and leaf and root extracts were more toxic than stem extracts. Dose and solvent of extraction significantly influenced mortality ($p < 0.05$) of all stages for methane and hexane extracts except for hexane root extracts ($p > 0.05$) used against L3s. Since calculated goodness of fit was greater than the critical value ($\chi^2 = 22.4$; df = 22; $p < 0.05$) for all cases, the null hypothesis was rejected and the conclusion that *U. massaica* crude extracts was toxic to immatures of *An. gambiae* in vitro was adopted. It is concluded that *U. massaica* crude extracts are toxic against immatures of *An. gambiae* in vitro.

Keywords *Urtica massaica*; Methanol; Hexane; Lethal effect; *Anopheles gambiae*

1 Background

Mosquitoes are vectors of global public health importance as the mosquito borne infections (MBI) (WHO, 2020) for which they are famous are of global public health concern (WHO, 2022). Indeed, the infections threaten more than 40% of the world's population (Franklinos et al., 2019), with malaria accounting for the highest reported cases of morbidity and mortality (Maharaj et al., 2019; WHO, 2021; Oladipo et al., 2022; Li et al., 2024). Most cases of malaria infections occur in sub-Saharan Africa (WHO, 2019; WHO, 2020). Intervention against malaria is largely through vector management due to lack of effective medication and vaccination and though RTS, S/AS01 vaccine has been endorsed against malaria (WHO, 2021, Ogieuhi et al., 2024), it is faced with a myriad of challenges (Sallam et al., 2025). Additionally, the vaccine is mainly meant for children and regions with moderate to high *Plasmodium falciparum* malaria transmission (WHO, 2021).

The first line mitigative measure against malaria vector over the years has been the use of synthetic insecticides on various platforms (WHO, 2018; WHO, 2019). These have successfully managed the vector densities (Derua et al., 2018; Derua et al., 2019), interfered with their host-seeking behaviour, reduced their contacts with humans and reduced malaria disease transmission (Cibulskis et al., 2016, Govindarajan et al., 2016). However, their continued indiscriminate use has led to resistance in mosquito populations (Oduola et al., 2019; WHO, 2020; Peng et al., 2022) in addition to unwarranted environmental toxicity (Deng et al., 2019; Semenza et al., 2022; Wafula et al., 2023). These challenges are expected to escalate with emerging issue with climate change, alien vector spp. and the ever-growing threat of resistance to antimalarial drugs and insecticides (Mordecai et al., 2020; Li et al., 2024). Envisaged solution is bringing on board new inventions and strategies (Richards et al., 2020).