IN VITRO AND *IN SILICO* CHARACTERIZATION OF THE ANTICHOLINESTERASE ACTIVITY OF SELECT TERPENOIDS AGAINST *ANOPHELES* VECTORS

ABSTRACT

Malaria is a life-threatening plasmodial disease that is transmitted by female *Anopheles* mosquitoes. Major African malaria vectors include *Anopheles arabiensis*, *An. funestus*, *An. gambiae* and *An. coluzzii*. Malaria vector control programs have shown effectiveness in reducing the *Anopheles* populations. The main insecticide classes used in these interventions include pyrethroids, organochlorines, organophosphates, carbamates, and neonicotinoids. Nevertheless, the development of *Anopheles* resistance to these insecticide classes has greatly reduced the effectiveness of these interventions. A common resistance mechanism is through rapid detoxification of insecticides by overexpressed P450 monooxygenases. Although acetylcholinesterase (AChE) is a valid target in *Anopheles* vector, current anticholinesterase insecticides suffer from resistance and low selectivity between insect and mammal AChE targets. This indicates the urgent need to discover novel AChE inhibitors with higher affinity to *Anopheles* AChE compared to the mammal target, and less prone to resistance caused by the overexpressed monooxygenases. Identification of novel AChE inhibitors from natural sources and their potential to kill *Anopheles* during all its different life stages, presents a cost-effective approach. This PhD study aimed to identify such novel AChE inhibitors from essential oil sources and assess them for consistent activity against *Anopheles* species with hyperactive P450 monooxygenases. In this study, molecular differences between *Anopheles* and human AChEs were identified showing the opportunity to develop selective *Anopheles* AChE inhibitors. A novel approach was used to integrate the *in silico* and *in vitro* assays in assessing the *Anopheles* AChE inhibitory potential of select terpenoids and coupled these to the *in vivo* assays against different life stages of *Anopheles*. The terpenoids, farnesol, (-)-α-bisabolol, *cis*-nerolidol, *trans*-nerolidol, and methyleugenol were identified as potent *Anopheles* AChE inhibitors and larvicidal agents with moderate adulticidal effects. Farnesol and (-)-α-bisabolol also displayed pupicidal activity, while methyleugenol inhibited the hatching of *Anopheles* eggs. Generally, farnesol and (-)-α-bisabolol were highly active across the *Anopheles* species, except in the strain with P450-based metabolic resistance. In contrast, the efficacy of *cis*-nerolidol, *trans*-nerolidol, and methyleugenol was not affected by this resistance mechanism. This research suggests that *cis*-nerolidol, *trans*-nerolidol, and methyleugenol are potential candidates for further development as anticholinesterase bioinsecticides.

Keywords: Malaria, *Anopheles*, life cycle, terpenoids, monooxygenases