UNIVERSITI TEKNOLOGI MARA

STYRYLLACTONES FROM ANTI-DENGUE ACTIVE FRACTION OF A *GONIOTHALAMUS LANCEOLATUS* LEAVES THROUGH *IN SILICO* AND *IN VITRO* EVALUATION

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Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Science)

Faculty of Applied Sciences

March 2024

ABSTRACT

Goniothalamus lanceolatus Miq. is an indigenous plant found in the rainforest of Sarawak. This ethnomedicinal plant has historically been used by native people as a mosquito repellent, to treat fevers, cancer, and abortion. The dichloromethane extract of leaves of *G. lanceolatus* (GLLD) showed promising results against DENV-2 with an IC_{50} value of 4.16 µg/mL, CC_{50} value of 24.23 µg/mL and a selectivity index (SI) of 5.82. This extract was selected for further fractionation to afford six fractions (F1-F6). These fractions were evaluated against DENV-2 (NGC strain) replication through plaque reduction assay. Based on the results, fraction F2 was identified as the most promising fraction against DENV-2 with an IC_{50} value of 1.62 µg/mL, CC_{50} value of 98.99 µg/mL, and a SI value of 61.10. Deep metabolome analysis of molecular networking highlighted that fraction F2 is mainly comprised of styryllactones and an itional 18 styryllactones were further annotated. Isolation and purification from this active anti-dengue fraction led to the characterization of eight styryllactones. All the structures were elucidated using 1D-NMR and 2D-NMR spectroscopy, whilst electronic circular dichroism data (ECD), NOESY experiments, and data comparison with literature values were used to establish the absolute configurations. The compounds were isolated during a bioactivity-directed comprising of two 2H-tetrahydropyran derivatives, (one new 3-*epi*-goniothalesdiol A **1** and known goniothalesdiol A **2**), one styryl-pyrone (goniodiol **3**), three pyrano-pyrone (1*S*,5*S*,7*R*,8*S*-3-*exo*,7-*endo*-(+)-8-*epi*-9-deoxygoniopypyrone **4**, deoxygoniopypyrone B **5**, and parvistone D **6**), one furano-pyrone ((+)-goniofupyrone B **7**), and one bis-styryllactone (6*S*,7*S*,8*S*,6*S*',7*S*',8*S*'-(+)-goniolanceolatin A **8**). The new compound **1** differs from the previously reported goniothalesdiol A in the absolute stereochemistry at position C-3 which was proposed as (2*R*,3*R*,4*S*,6*R*) and was named as 3-*epi*-goniothalesdiol A. Interestingly, C-4 from pyrano moiety of **1** originated from C-6 of rare *S*-goniothalamin reported from *G. lanceolatus*. *In vitro* evaluation showed that compound **8** had a promising anti-dengue viral activity with the highest SI value of 4.06 and by qRT-PCR with an SI value of 5.30. The findings from *in silico* studies suggest that compound **8** has the potential to act as an inhibitor against the Envelope (E), NS5 methyltransferase, and NS5 RdRp proteins of DENV.

ACKNOWLEDGEMENT

First and foremost, I would like to praise Allah the Almighty, the Most Gracious, and the Most Merciful for His blessing for giving me strength and patience in completing this Ph.D. journey. May the peace, blessings, and salutations of Allah be upon our noble Messenger, Muhammad, and upon his family and his Companions.

My utmost gratitude goes to my supervisor Prof. Dr. Nor Hadiani Ismail for the guidance, knowledge, advice, motivation, and opportunity from the day I enrolled in my Ph.D. course till now. It was a great honour to work under her supervision. I would like to thank my co-supervisor Dr. Syahrul Imran Abu Bakar and Dr. Lam Kok Wai for the consultation, advice, and knowledge. Not forgetting, Dr Murizal Bin Zainol and Dr Adlin Afzan from Herbal Medicine Research Centre, Institute of Medical Research for guidance and for giving me the opportunity to use the facilities to carry out my biological activity assay.

Finally, this PhD degree is dedicated to my father, Abdullah bin Jantan and my mother, whom I love with all my heart and I owe them tremendously for their unceasing support, prayers, motivation, advice, and encouragement throughout completing the study. Thank you for allowing me to make my dream come true. Million thanks to my beloved siblings; Nor Arlita, Ahmad Nizam, Nor Arnida Ira, Nor Hafazah, and Ahmad Shafiq, and to all my lovely nephews and nieces for cheering me along the journey.

Many thanks go to all the staff and fellows at Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns) for their support and knowledge sharing. Special thanks to the lectures; Dr. Nurulfazlina Edayah, Dr. Najah, Dr. Vicky, Dr. Amalina, and Dr. Noraini, and all my lab mates; Nor Syaidatul, Kamsirah, Hidayatul Atiqah, Afiqah, Anis, Sabrina, Bazlah and all my friends at AuRIns, thank you for standing by my side when the things get hard, and thank you for the opinion, knowledge sharing and ideas along this journey. May Allah bless all of you.

Last but not least, my gratitude also goes to the Ministry of Higher Education and Universiti Teknologi MARA for the research grants of [UiTM600-RMC/FRGS 5/3 (106/2021)], [UiTM600-IRMI/FRGS 5/3 (111/2019)] and [FRGS/1/2016/STG01/UITM/01/1].

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Dengue fever has been of major concern to governments and the World Health Organization (WHO) because it affects mortality and morbidity around the world, specifically in the tropics and subtropical regions. About half of the world's population is presently at risk from infection, and it is believed that there are between 100-400 million cases of dengue worldwide every year. According to the Ministry of Health (MOH) Malaysia (2022), dengue fever has the highest prevalence rate among communicable diseases in Malaysia, with 397 cases per 100,000 population. Despite intensive biomedical studies, currently, there are no direct-acting antivirals to combat dengue virus (DENV) and the treatment continues to rely on supportive measures like fluid replacement and analgesic use (Lee et al., 2023). Indisputably, novel anti-DENV inhibitors need to be designed and developed to counter this problem (Paz-Bailey et al., 2021).

DENV is a member of the family Flaviviridae and this flavivirus carries a positive sense with single-stranded RNA viruses with approximately 55 nm in diameter (Paranjape & Harris 2010). Non-structural proteins play a major role in the evasion of innate immune responses, virion assembly, and genome replication. Especially envelope (E) and NS5 proteins are crucial for the initial attachment of viral particles to host cell receptors and the formation of the viral particle during the infection cycle. The development of an effective therapeutic agent against DENV protein is crucial (Lee et al., 2023).

Natural products (NPs) are a robust source of new drug leads in combating emerging diseases in humans. A recent release of the medicine data compiled by the US Food and Drug Administration (FDA) revealed that 67% of the 1562 small molecules approved in the market between 1981 and 2014 comprised natural products (Newman et al., 2016). Nowadays, many treatments use medicinal plants to inhibit virus replication at various stages (Saleh et al., 2020). Unfortunately, only a handful of research has been done on potential plants against the DENV. Numerous plants belonging to the Lamiaceae family are used for the treatment of DENV infection by