Malaysian Journal of Microbiology, Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Comparative screening of anti-dengue activity in aqueous and ethanol extracts of mangrove plants from Sabah

Mayrlidzatul Farhain Misrah[1], Nur Athirah Yusof [1], Zarina Amin[1], Kholis Abdurachim Audah[2] and Ruzaidi Azli Mohd Mokhtar[1]*

[1]Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu Sabah, Malaysia.
[2]Department of Biomedical Engineering, Swiss German University, Tangerang 15143, Indonesia.
Email: ruzaidi@ums.edu.my

ABSTRACT

Aims: Dengue virus is a global pathogen that lacks an effective vaccine or therapy. Screening medicinal plants for anti-dengue properties provides a promising avenue to identify potent compounds. Mangroves, known for their resilience in harsh conditions, produce a diverse range of natural products with unique biochemical profiles, which hold potential for anti-dengue treatments. This study aims to evaluate the anti-dengue activity of selected mangrove plant species from Sabah against DENV2 NS2B-NS3pro, utilizing an enzymatic protease assay.
Methodology and results: Six mangrove species (*Avicennia marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Rhizophora apiculata*, *Rhizophora mucronata* and *Xylocarpus granatum*) were investigated, with various plant parts subjected to aqueous and ethanol extraction. The results demonstrated significant anti-dengue activity in both aqueous and ethanolic extracts of the mangroves against DENV2 NS2B-NS3pro, with IC_{50} values ranging from 0.95 µg/mL to 6.24 µg/mL. Notably, the ethanolic extract of *R. apiculata* leaves exhibited the highest inhibition, with an IC_{50} value of 0.95 µg/mL.
Conclusion, significance and impact of study: These findings suggest that the ethanolic extracts from *R. apiculata* leaves hold promise as potential candidates for dengue treatment. This study underscores the importance of natural products as valuable sources for the development of novel anti-dengue treatments, highlighting the need to explore mangroves in the quest for effective therapeutic options.

Keywords: Anti-dengue, dengue virus, DENV2 NS2B-NS3pro, mangrove, *rhizophora apiculata*

INTRODUCTION

Dengue, a viral disease transmitted through the bites of infected Aedes mosquitoes, is a major concern (Alobuia *et al.*, 2015). The primary serotypes responsible for dengue infection are DENV-1, DENV-2, DENV-3 and DENV-4, and individuals can face up to four infections in their lifetime. However, the risk of severe dengue increases with secondary infection by different serotypes (Weaver and Vasilakis, 2009; Nitsche *et al.*, 2014). The global incidence of dengue has surged in recent decades; reported cases to WHO escalated from 505,430 in 2000 to 5.2 million in 2019 (WHO, 2023). Notably, Asian countries accounted for the majority of cases in 2019, with the Philippines (420,000), Vietnam (320,000) and Malaysia (131,000) being most affected (WHO, 2023). Despite the substantial case numbers, a specific cure for dengue remains elusive (Harapan *et al.*, 2020). Furthermore, the challenge of antibody-dependent enhancement (ADE) has hindered dengue vaccine development (Shukla *et al.*, 2020).

Dengue virus belongs to the genus flavivirus of the Flaviviridae family (Murugesan and Manoharan, 2020). The genome encodes three structural proteins which are the capsid (C), envelope (E) and membrane (M) proteins and seven non-structural proteins which are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 by the host proteases (Furin and signalase) and the two-component viral NS2B-NS3 protease (Sundar *et al.*, 2022). The DENV NS2B-NS3 pro (serine protease), which belongs to the trypsin superfamily, contains a catalytic triad in its active site formed by His51, Asp75 and Ser135 residues (Li *et al.*, 2017). The N-terminal domain of NS3 houses NS3 protease, which, together with 40 residues (hydrophilic) of NS2B, exhibits the serine protease activity (Wahaab *et al.*, 2021). The lack of this NS2B-NS3 interaction renders the NS3 protease less active or inactive. Further, this NS2B-NS3 alignment forms the S2 and S3 sub-pockets in the protease active site. Since viral replication and propagation depend on the activity of DENV NS2B-NS3 pro, the complex serves as an attractive target for the antiviral drug design against DENV (Murtuja *et al.*, 2021).

***Corresponding author**

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

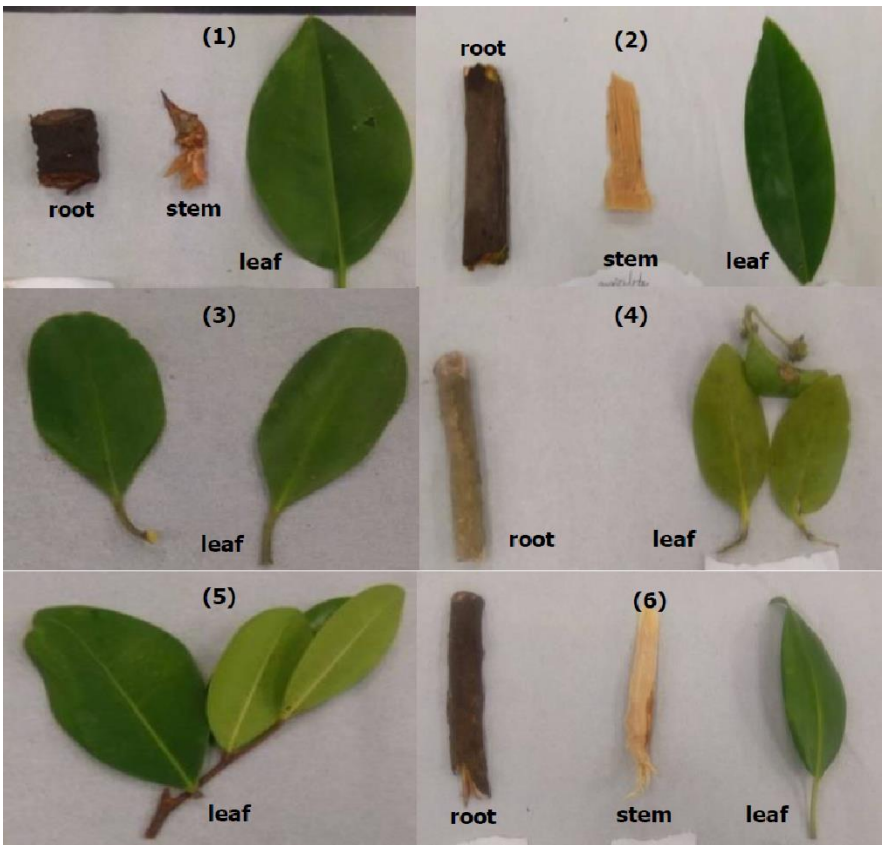

Figure 1: Local mangrove species used in this study including (1) *R. mucronata*, (2) *R. apiculata*, (3) *C. tagal*, (4) *A. marina*, (5) *R. apiculata* and (6) *B. gymnorrhiza*.

Given the high dengue incidence, it becomes imperative to explore alternative approaches to combat the disease. Medicinal plants offer a potential alternative treatment to alleviate this burden. Throughout history, plants have inspired the discovery of novel drug compounds, contributing significantly to human health (Thomford *et al.*, 2018). Among plant bioactive compounds, polyphenols, essential secondary metabolites, emerge as natural antioxidants protecting against human diseases (Cory *et al.*, 2018). With concerns about antibiotic resistance, researchers are turning to plant extracts and active compounds, which are considered safer and less toxic (Michiels *et al.*, 2012).

Mangroves, highly productive ecosystems, hold promise due to their capacity to produce specific compounds for survival in challenging environments. In Malaysia, Sabah boasts the largest mangrove forest area. Yet, their potential in combating diseases, especially among Sabah's indigenous people, like dengue, remains underexplored. Phytochemical studies have revealed mangroves as rich sources of active compounds, including phenolics, with therapeutic applications against pathogens and viral diseases (Cáceres *et al.*, 2020; Gomes *et al.*, 2020). They also exhibit antibacterial, antiviral, antifungal, antimalarial and anti-inflammatory properties, making them potential sources of mosquito larvicides (Amudha *et al.*, 2014; Meenakshi and Jayaprakash, 2014). Hence, mangroves offer promising candidates for the search for new agents against dengue, harboring diverse biologically active compounds (Dahibhate *et al.*, 2020). This study aims to investigate the inhibitory potential of mangrove extracts against the dengue virus, considering these properties.

MATERIALS AND METHODS

Plant sampling and species collection

Six species of mangroves (*A. marina*, *B. gymnorrhiza*, *C. tagal*, *R. apiculata*, *R. mucronata* and *X. granatum*) were randomly collected from various parts of the leaves, stems and roots (Figure 1) within the Sulaman Lake Mangrove Sanctuary in Tuaran, Sabah, Malaysia. The identification of the collected plants was carried out by Mr. Johnny Gisil, a botanist from the Biology Tropical and Conservation Institute at Universiti Malaysia Sabah, Malaysia. The selection of mangrove species and plant parts for this study adhered to Audah *et al.* (2020) and was based on the availability of all samples present in Sulaman Lake Mangrove Sanctuary, Tuaran. To prepare the collected samples, a thorough cleansing process was initiated, involving washing with tap water followed by

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

thorough rinsing with distilled water. Subsequently, the samples were placed in an oven set at 40 °C for drying. The resulting dried samples were finely ground into a powder using a blender, stored in 50 mL falcon tubes, and kept at a temperature of 4 °C until the subsequent solvent extraction process.

Preparation of an aqueous extract

The powdered plant samples were soaked in distilled water (maceration method) in a ratio of 1:10 (1 g in 10 mL of solvent) and allowed to be stirred overnight using a magnetic stirrer. The solutions were first passed through a muslin cloth, and the filtrate collected was filtered again using Whatman's filter paper No.1 to achieve a particle-free solution. The extract was then freeze-dried until completely dried (approximately 48 h) and stored at -80 °C until further analysis.

Preparation of ethanolic extract

The powdered plant samples were soaked in 70% ethanol (R&M Chemicals, Malaysia) in a ratio of 1:10 (1 g in 10 mL of solvent) and allowed to be stirred overnight using a magnetic stirrer. The solutions were first passed through a muslin cloth and the filtrate collected was filtered again using Whatman's filter paper No. 1. The filtrate was evaporated using a rotary evaporator (Buchi R-210, Switzerland) to remove the solvent and to obtain a concentrated extract. The extract was then freeze-dried until completely dried (approximately 48 h) and stored at -80 °C until further analysis.

Expression and purification of DENV2 NS2B-NS3pro

The full-length DENV2 NS2B-NS3pro gene was synthesized by inserting synthetic cDNA into plasmid vector pET15b, incorporating a 6His-tag at the N-terminal, via *Nde*I-*Bam*HI sites. This synthesis was outsourced to Apical Scientific Sdn. Bhd. Transformation of the expression plasmid was done in *E. coli* BL21(DE3) cells using the heat-shock method. The transformed NS2B-NS3pro was expressed by inducing with isopropyl β-d-1-thiogalactopyranoside (IPTG, Nacalai Tesque, Japan), followed by cell lysis and protein purification using Nickel-Nitrilotriacetic Acid (Ni-NTA) chromatography. The purified protein was analyzed through 12% SDS-PAGE. Further analysis and storage were conducted for subsequent experiments.

Anti-dengue screening by protease inhibition assay

The antiviral activity of mangrove extracts against DENV2 NS2B-NS3pro was performed using the following method by Rothan *et al.* (2014). In the present study, the concentration of extracts used was (12.5, 25, 50, 100, 200 and 400) µg/mL while the enzyme concentration was 50 µg/mL. On the other hand, the positive control wells contained Tris buffer (200 mM, pH 8.5) (R&M Chemicals, Malaysia), enzyme and substrate, while the negative control wells contained tris buffer, extract and substrate. Aprotinin (Sigma-Aldrich, USA) was used as a standard, and the assay was performed in a microplate 96-well with a total reaction volume of 200 µL.

The mixture of enzyme and extract was initially prepared in the Tris buffer (200 mM, pH 8.5). Then, the mixtures were incubated for 15 min at 37 °C. Next, 10 µM of fluorogenic substrate t-Butyloxycarbonyl-Glycyl-L-Arginyl-L-Arginine4-Methyl-Coumaryl-7-Amide (Boc-Gly-Arg-Arg-AMC) was added into the mixtures and further incubated at the same temperature for another 1 h. After 1 h of incubation, the reaction mixtures were measured spectrophotometrically using a fluorescence emission at 465 nm following excitation at 385 nm. All samples were done in triplicate. The IC_{50} value, which is the concentration of extract needed to inhibit 50% of enzyme activity, can be calculated from the calibration curve by linear regression. The inhibitory activity of each extract was calculated according to the following formula:

$$\text{Inhibition (\%)} = [1 - (A_t - A_n)/(A_p - A_b)] \times 100$$

Where, A_b is the absorbance of a blank, A_t is the absorbance of a test sample, A_p is the absorbance of a positive control and A_n is the absorbance of a negative control.

Phytochemical screening using liquid chromatography mass spectroscopy

The extract that exhibited the highest inhibition of anti-dengue activity was further analyzed using Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS/MS) from the Bruker UHPLC Elute LC Chromatography System, US. The extracts were prepared according to a modified method previously described (Mondal *et al.*, 2014). A stock sample solution (1 mg/mL) was prepared by dissolving 1 mg of extract in 1 mL of HPLC grade methanol (Merck, USA). Then, 100 µL of the stock solution was further dissolved in 900 µL of HPLC grade methanol to obtain the final concentration of extract at 100 µg/mL. The sample was filtered through a 0.2 µm Nylon syringe filter to get a completely clear sample solution. Then, 500 µL of sample solution was transferred to autosampler vials to conduct the LC-Q-TOF-MS/MS analysis.

The chromatographic separation was carried out using the SB-C18 column (4.6 × 150 mm) with a 2.7 µm particle size at 30 °C. The mobile phases were with water 0.1% formic acid (A) and acetonitrile in water with 0.1% formic acid (B) at a flow rate of 0.3 mL/min. Analysis was performed using the following gradient elution: 0-40% B in 10 min, 40-20% B in 15 min, 20% B in 20 min, 20-100% B in 21 min, 100-0% B in 25 min. The volume of the sample injected was 5 µL. The raw data were processed using TASQ 1.5 Software Packages (Bruker Daltonics, Bremen, Germany).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

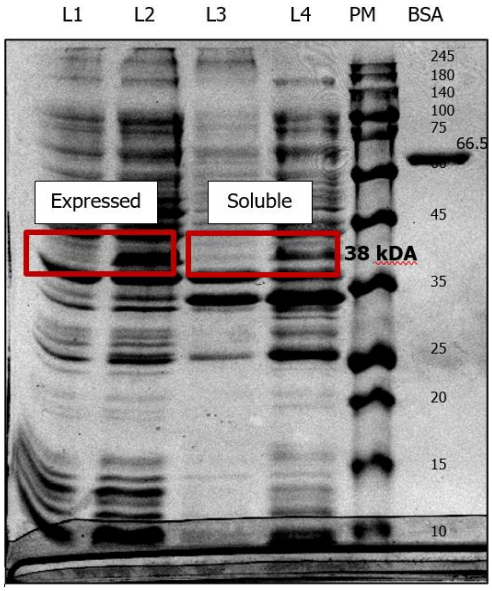

Figure 2: Expression of DENV2 NS2B-NS3pro. Abbrv: L1: Before IPTG induction, L2: After IPTG induction, L3: Pellet, L4: Supernatant, PM (Protein Marker), BSA (Bovine Serum Albumin, standard protein).

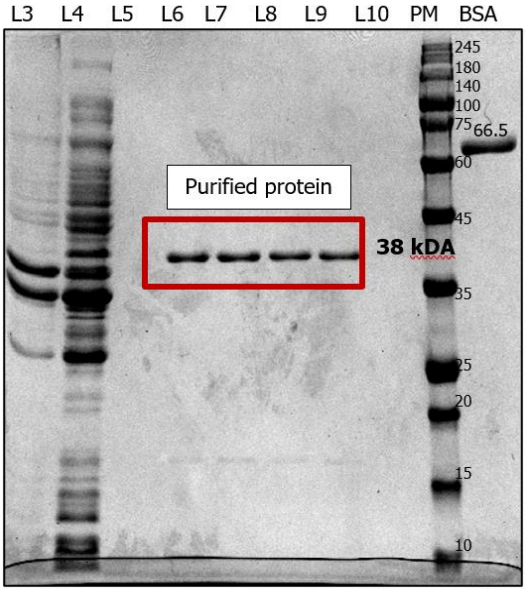

Figure 3: Purification of DENV2 NS2B-NS3pro. Abbrv: L3: Pellet, L4: Supernatant, L5 to L10: Aliquot protein, PM (Protein Marker), BSA (Bovine Serum Albumin, standard protein).

Statistical analysis

Statistical analysis was performed using IBM SPSS version 28.0 (IBM Co., USA) of one-way ANOVA, followed by the Tukey test for multiple comparisons of samples. A *p*-value less than 0.05 (typically ≤0.05) was considered significant.

RESULTS

Anti-dengue activity

Prior to the anti-dengue screening, the purified protein sample was obtained. Based on the SDS-PAGE analysis (Figure 2), the DENV2 NS2B-NS3pro was expressed at the expected size of 38 kDa upon incubation for 16 h in the presence of 0.5 mM IPTG, predominantly as a soluble protein as there is no fraction at the pellet. As shown in Figure 3, the DENV2 NS2B-NS3pro was purified with >95% purity by a single-step chromatography on Ni^{2+}-metal chelate affinity columns in the presence of an elution buffer containing 500 mM imidazole.

The inhibitory activity of mangrove extracts against DENV2 NS2B-NS3pro was quantified by determining the IC_{50} values (µg/mL). Both aqueous and ethanolic extracts from various mangroves demonstrated anti-dengue activity against DENV2 NS2B-NS3pro, as outlined in Table 1. Specifically, IC_{50} values for aqueous extracts ranged from 1.09 µg/mL to 6.24 µg/mL, while for ethanolic extracts, the range was 0.95 µg/mL to 4.02 µg/mL.

Notably, the ethanolic extract of *R. apiculata* leaves exhibited the highest inhibitory activity against DENV2 NS2B-NS3pro, demonstrating a promising IC_{50} value of 0.95 ± 0.27 µg/mL. Following closely were the aqueous extract of *R. mucronata* leaves (IC_{50}: 1.09 ± 0.14 µg/mL) and the ethanolic extract of *R. mucronata* leaves (IC_{50}: 2.27 ± 0.84 µg/mL).

In contrast, the inhibitory activity of aqueous extracts from *A. marina* roots, *R. apiculata* roots and *X. granatum* leaves were insufficient to determine IC_{50} values, as it fell below 50%. This suggests a limitation in the inhibitory potential of these extracts using the aqueous solvent.

In addition, the results emphasize the superior anti-dengue activity of ethanolic extracts compared to their aqueous counterparts. Notably, the IC_{50} value of the ethanolic extract of *R. apiculata* leaves differed significantly from all other samples, underscoring its exceptional potency against DENV2 NS2B-NS3pro. These findings highlight the potential of mangrove-derived ethanolic extracts as promising candidates in combating dengue infections.

Phytochemical analysis

In this study, we focused on the ethanolic extract of *R. apiculata* leaves, selecting it for in-depth phytochemical analysis due to its exceptional anti-dengue activity, as demonstrated in our previous assays. Our objective was to identify specific compounds present in this extract that might be responsible for its potent inhibitory effects against DENV2 NS2B-NS3pro. Figure 4 displays the results of our phytochemical screening, revealing the presence of several distinct compounds. The chromatogram exhibited peaks corresponding to specific

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

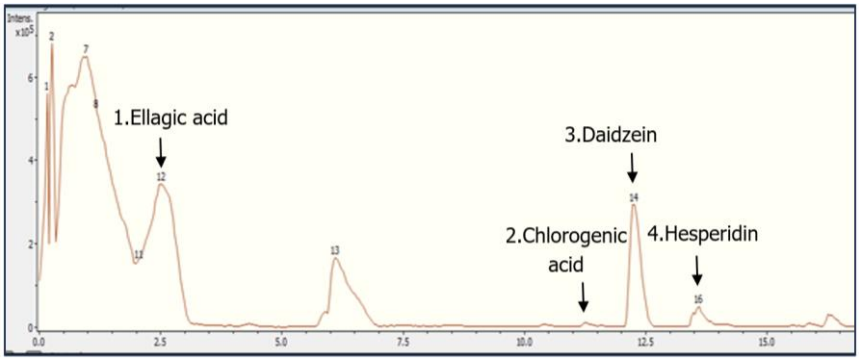

Figure 4: LC-QTOF-MS/MS chromatogram of ethanolic extract of *R. apiculata* leaves.

Table 1: Protease inhibition assay by IC_{50} value (µg/mL).

Enzyme	Extracts	IC_{50} value (µg/mL)	
		Aqueous	70% ethanol
DENV2 NS2B-NS3pro	AML	5.69 ± 0.55^{l}	3.10 ± 0.36^{e}
	AMR	CBD	4.02 ± 0.28^{k}
	BGL	4.37 ± 0.32^{k}	3.40 ± 032^{i}
	BGS	4.74 ± 0.87^{k}	3.37 ± 0.26^{h}
	BGR	6.24 ± 0.21^{m}	3.26 ± 0.25^{e}
	CTL	3.11 ± 0.61^{e}	2.82 ± 0.12^{d}
	RAL	3.80 ± 0.83^{j}	0.95 ± 0.27^{a}
	RAS	4.67 ± 0.79^{k}	3.55 ± 0.33^{j}
	RAR	CBD	3.40 ± 0.47^{i}
	RML	1.09 ± 0.14^{b}	2.27 ± 0.84^{c}
	RMS	3.38 ± 0.23^{h}	3.33 ± 0.20^{g}
	RMR	4.72 ± 0.49^{k}	3.09 ± 0.36^{e}
	XGL	CBD	3.30 ± 0.10^{f}

The values represent the means ± standard deviations of three replicates. Different letters indicate significant differences between samples (One-Way ANOVA, Duncan's Multiple comparison test, $P<0.05$). CBD = cannot be determined as the inhibitory activity is less than 50% inhibition.
Abbrv: AML, *Avicennia marina* leaves; AMR, *Avicennia marina* root; BGL, *Bruguierra gymnorrhiza* leaves; BGS, *Bruguierra gymnorrhiza* stem; BGR, *Bruguierra gymnorrhiza* root; CTL, *Ceriops tagal* leaves; RAL, *Rhizophora apiculata* leaves; RAS, *Rhizophora apiculata* stem; RAR, *Rhizophora apiculata* root; RML, *Rhizophora mucronata* leaves; RMS, *Rhizophora mucronata* stem; RMR, *Rhizophora mucronata* root; XGL, *Xylocarpus granatum* leaves.

compounds: 1. ellagic acid detected at 2.5 min, 2. chlorogenic acid observed at 11.3 min, 3. daidzein at 12.3 min and 4. hesperidin appearing at 13.6 min.

DISCUSSION

The noteworthy anti-dengue effects observed in both aqueous and ethanolic mangrove extracts underscore their potential as valuable sources of anti-dengue agents. Remarkably, the ethanolic extract from *R. apiculata* leaves exhibited the most potent anti-dengue effect among all extracts investigated. This highlights the likelihood that these extracts encompass active compounds proficient in countering DENV2 NS2B-NS3pro.

The mechanism behind the inhibition of DENV2 NS2B-NS3pro by these extracts can be elucidated through competitive inhibition. In this process, the extract competes with the substrate for binding at the enzyme's active site (Figure 5). Once the extract binds to the enzyme's active site, it disrupts the interaction between the substrate and enzyme, leading to a reduction in enzyme activity. Consequently, both aqueous and ethanolic extracts possess the capability to bind to and neutralize elements of DENV, potentially thwarting its adverse effects.

The well-established correlation between polyphenols, their antioxidant properties and their capacity to combat dengue is noteworthy (Loaiza-Cano *et al.*, 2020). These compounds can obstruct various stages of the dengue virus life cycle through diverse mechanisms, including impeding viral attachment, retarding early replication, mitigating viral impact, and interfering with viral binding (Zandi *et al.*, 2011). This multifaceted strategy curtails the virus's ability to propagate and cause harm. The potential of antioxidants as treatments for viral infections has captured researchers' attention. Additionally, indications suggest that mosquitoes may harness antioxidant defenses to guard against viruses (Chen *et al.*, 2011).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 671-677
DOI: http://dx.doi.org/10.21161/mjm.230012

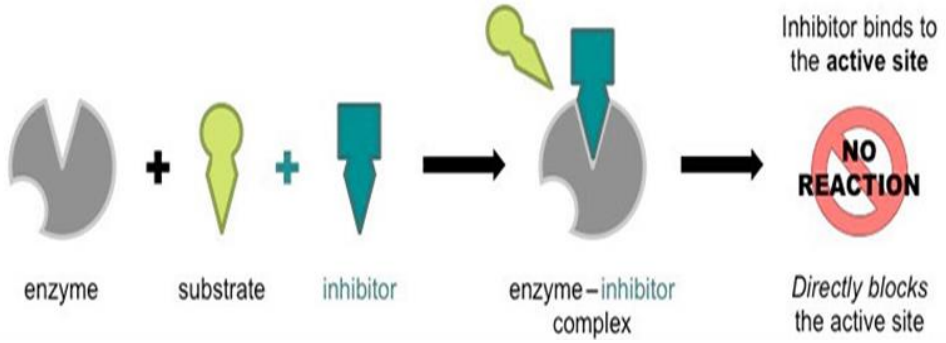

Figure 5: Competitive inhibition of enzyme activity. Competitive inhibitors compete with the substrate for the active site of the enzyme and form an enzyme-substrate complex.

Intriguingly, Chandrasena *et al.* (2014) hinted at a connection between oxidative stress and the severe phase of dengue infection, implying that antioxidants might mitigate the virus's impact. The observed anti-dengue activity in these mangrove plants could indeed be attributed to their polyphenol and antioxidant compounds, working in tandem to combat DENV2 NS2B-NS3pro. A prior study from our team found that the ethanolic extract from *R. apiculata* leaves exhibited strong antioxidant activity, with an antioxidant value of 23.21 µg/mL, underscoring its robust antioxidant potential (Mohd Mokhtar *et al.*, 2022). This substantial antioxidant capability could be contributing to the potent anti-dengue effect of the ethanolic extract from *R. apiculata* leaves.

In summary, our study underscores the potential of mangrove extracts as promising anti-dengue agents. The observed anti-dengue effects in both aqueous and ethanolic extracts, particularly the ethanolic extract from *R. apiculata* leaves, necessitate further investigation into their mechanisms against DENV2 NS2B-NS3pro. The presence of polyphenol and antioxidant compounds further strengthens their potential as potent antiviral agents, warranting in-depth exploration of their efficacy against dengue infections and their suitability for therapeutic use.

Furthermore, the ethanolic extract from *R. apiculata* leaves, displaying the highest anti-dengue potency, underwent chemical analysis to reveal its composition. This analysis unveiled the presence of compounds such as ellagic acid, chlorogenic acid, daidzein and hesperidin. Notably, prior research suggests that some of these compounds, such as ellagic acid and daidzein, also possess anti-dengue capabilities (Zandi *et al.*, 2011; Bupesh *et al.*, 2014). This could elucidate the heightened anti-dengue potential of this particular extract. This insight implies that the collective action of these compounds within the extract contributes to its formidable anti-dengue efficacy.

Altogether, the identification of ellagic acid, chlorogenic acid, daidzein, and hesperidin within the ethanolic extract of *R. apiculata* leaves sheds light on the basis for its robust anti-dengue effectiveness. This alignment with prior research underscores the role of these compounds in countering dengue infections. This discovery enriches our comprehension of the extract's potency and accentuates the significance of specific compounds in the overall antiviral action of mangrove extracts. This insight encourages further exploration of their mechanisms and potential for dengue treatment.

CONCLUSION

In conclusion, this study affirms that all the tested mangrove extracts possess anti-dengue activity. Among the various samples assessed, the ethanolic extracts from *R. apiculata* leaves stand out as the most potent contenders for potential anti-dengue agents. This research underscores the considerable promise of mangroves as sources of anti-dengue solutions. Moreover, the phytochemical analysis provides evidence supporting the notion that mangroves could yield compounds with the potential to contribute to future anti-dengue treatments.

ACKNOWLEDGEMENTS

This research was supported by the External Collaboration Research Grant Scheme (GKP-0021-2018) and UMS Postgraduate Research Grant (UMSgreat).

CONFLICTS OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES

Alobuia, W. M., Missikpode, C., Aung, M. and Jolly, P. E. (2015). Knowledge, attitude and practices regarding vector borne disease in Western Jamaica. *Annals of Global Health* **81(7), 654-663.**

Amudha, Prabuseenivasan and Kumar, V. (2014). Antimycobacterial activity of certain mangrove plants against multi-drug resistant *Mycobacterium tuberculosis*. *Asian Journal of Medical Sciences* **5, 54-57.**

Audah, K. A., Batubara, R., Julkipli, W., Wijaya, E., Kurniawaty, E. and Batubara, I. (2020). Antibacterial

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

screening of mangrove extract library showed potential activity against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Tropical Life Science* **10(2), 105-111.**

Bupesh, G., Raja, R. S., Saravanamurali, K., Kumar, V. S., Saran, N., Kumar, M. *et al.* (2014). Antiviral activity of ellagic acid against envelope proteins from dengue virus through *in silico* docking. *International Journal of Drug Development and Research* **6(2), 205-210.**

Cáceres, M., Hidalgo, W., Stashenko, E., Torres, R. and Ortiz, C. (2020). Essential oils of aromatic plants with antibacterial, anti-biofilm and anti-quorum sensing activities against pathogenic bacteria. *Antibiotics* **9(4), 147.**

Chandrasena, L. G., Peiris, H., Kamani, J., Wanigasuriya, P., Jayaratne, S. D., Wijayasiri, W. A. *et al.* (2014). Antioxidants in patients with dengue viral infection. *Southeast Asian Journal of Tropical Medicine and Public Health* **45(5), 1015-1022.**

Chen, T. H., Tang, P., Yang, C. F., Kao, L. H., Lo, Y. P., Chuang, C. K. *et al.* (2011). Antioxidant defense is one of the mechanisms by which mosquito cells survive dengue 2 viral infection. *Virology* **410(2), 410-417.**

Cory, H., Passarelli, S., Szeto, J., Tamez, M. and Mattei, J. (2018). The role of polyphenols in human health and food systems: A mini-review. *Frontiers in Nutrition* **5, 87.**

Dahibhate, N. L., Roy, U. and Kumar, K. (2020). Phytochemical screening, antimicrobial and antioxidative activities of selected mangrove species. *Current Bioactive Compounds* **16(2), 152-163.**

Gomes, F., Dias, M. I., Lima, Â., Barros, L., Rodrigues, M. E., Ferreira, I. C. *et al.* (2020). *Satureja montana* L. and *Origanum majorana* L. decoctions: Antimicrobial activity, mode of action and phenolic characterization. *Antibiotics* **9(6), 294.**

Harapan, H., Michie, A., Sasmono, R. T. and Imrie, A. (2020). Dengue: A mini review. *Viruses* **12(8), 829.**

Li, Z., Zhang, J. and Li, H. (2017). *Flavivirus* NS2B/NS3 protease: Structure, function, and inhibition. *In*: Viral Proteases and Their Inhibitors. Gupta, S. P. (eds.). Elsevier, United States. **pp.163-188.**

Loaiza-Cano, V., Monsalve-Escudero, L. M., Filho, C. D. S. M. B., Martinez-Gutierrez, M. and Sousa, D. P. (2020). Antiviral role of phenolic compounds against dengue virus: A review. *Biomolecules* **11(1), 11.**

Meenakshi, S. V. and Jayaprakash, K. (2014). Mosquito larvicidal efficacy of leaf extract from mangrove plant *Rhizophora mucronata* (Family: Rhizophoraceae) against *Anopheles* and *Aedes* species. *Journal of Pharmacognosy and Phytochemistry* **3(1), 78-83.**

Michiels, J. A., Kevers, C., Pincemail, J., Defraigne, J. O. and Dommes, J. (2012). Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chemistry* **130(4), 986-993.**

Mohd Mokhtar, R. A., Misrah, M. F., Kansil, T., Amin, Z., Yusof, N. A. and Budiman, C. (2022). Evaluation of antioxidant activity and total phenolics of selected mangrove plants in Sabah. *Borneo International Journal of Biotechnology* **2, 14-21.**

Mondal, S., Majumdar, S., Geetha, V., Gadamsetty, G., Lakshmipathy, R., Sheela, A. *et al.* (2014). GC-MS and LC-MS analysis of ethanolic extract of *Drypetes sepiaria*. *Malaya Journal of Biosciences* **1(4), 207-213.**

Murtuja, S., Shilkar, D., Sarkar, B., Sinha, B. N. and Jayaprakash, V. (2021). A short survey of dengue protease inhibitor development in the past 6 years (2015-2020) with an emphasis on similarities between DENV and SARS-CoV-2 proteases. *Bioorganic and Medicinal Chemistry* **49, 116415.**

Murugesan, A. and Manoharan, M. (2020). Dengue virus. *In*: Emerging and Reemerging Viral Pathogens. Ennaji, M. M. (eds.). Academic Press, United States. **pp. 281-359.**

Nitsche, C., Holloway, S., Schirmeister, T. and Klein, C. D. (2014). Biochemistry and medicinal chemistry of the dengue virus protease. *Chemical Reviews* **114(21), 11348-11381.**

Rothan, H. A., Zulqarnain, M., Ammar, Y. A. S., Tan, E. C., Rahman, N. A. and Yusof, R. (2014). Screening of antiviral activities in medicinal plants extracts against dengue virus using dengue NS2B-NS3 protease assay. *Tropical Biomedicine* **31, 286-296.**

Shukla, R., Ramasamy, V., Shanmugam, R. K., Ahuja, R. and Khanna, N. (2020). Antibody-dependent enhancement: A challenge for developing a safe dengue vaccine. *Frontiers in Cellular and Infection Microbiology* **10, 572681.**

Sundar, S., Piramanayagam, S. and Natarajan, J. (2022). A review on structural genomics approach applied for drug discovery against three vector-borne viral diseases: Dengue, Chikungunya and Zika. *Virus Genes* **58(1), 151-171.**

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A. *et al.* (2018). Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. *International Journal of Molecular Sciences* **19(6), 1578.**

Wahaab, A., Mustafa, B. E., Hameed, M., Stevenson, N. J., Anwar, M. N., Liu, K. *et al.* (2021). Potential role of flavivirus NS2B-NS3 proteases in viral pathogenesis and anti-flavivirus drug discovery employing animal cells and models: A review. *Viruses* **14(1), 44.**

Weaver, S. C. and Vasilakis, N. (2009). Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infection, Genetics and Evolution* **9(4), 523-540.**

WHO, World Health Organization. (2023). Dengue and severe dengue. WHO: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue [Retrieved on 17 March 2023].

Zandi, K., Teoh, B. T., Sam, S. S., Wong, P. F.,Mustafa, M. R. and Abubakar, S. (2011). Antiviral activity of four types of bioflavonoid against dengue virus type 2. *Virology Journal* **8, 560.**