

Dengue protease inhibition activity of selected Malaysian medicinal herbs

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Abstract. Dengue fever is one of major health problem around the world including Malaysia. It is caused by the arthropode-borne flavivirus and transmitted by the bite of the *Aedes aegypti* or *Aedes albopictus* mosquito infected with one of the four dengue virus serotypes (DENV-1, DENV-2, DENV-3, or DENV-4). In this study, a screening exercise of various Malaysian medicinal plants showed that the extracts of *Lawsonia inermis*, *Dryobalanops aromatica*, *Punica granatum*, *Zizyphus jujuba* Lam. and *Zingiber zerumbet* exhibited potent inhibitory activity against NS2B-NS3 serine protease. The methanol extracts of *Dryobalanops aromatica* showed inhibition of 99.70 % at concentration of 200 µg/mL with IC₅₀ value of 0.30 ± 0.16 µg/mL.

INTRODUCTION

Dengue is a mosquito-borne viral disease and classified as one of the rapidly spread diseases in the world with about 4.54 million dengue infections worldwide in 2016 (World Health Organization, 2007a). Dengue can be transmitted through the saliva of the female mosquito of *Aedes aegypti* and *Aedes albopictus*. According to the World Health Organization (WHO), it was estimated that 2.5 billion people, two-fifths of the world's population, are susceptible to dengue infection risk. The organization reported increase of 55% of cases from the year of 2010 (2.2 million) to 2015 (3.2 million) (World Health Organization, 2007a). In Malaysia, even though dengue infection cases

decreased by 25.3% from January until May 2017 as compared to the same period in 2016, it is still considered to be one of the fatal health threats to Malaysia (World Health Organization, 2007b). Therefore, there is an urgent need to look for effective modes to prevent and cure the disease.

Recently, Sanofi had successfully developed the first WHO-approved vaccine against dengue known as Dengvaxia. It was claimed to be effective against all of the four different dengue serotypes. Nonetheless, the effort of finding potential therapeutics against dengue infection continues as the efficiency of Dengvaxia varies for the different serotypes, with the lowest efficiency was shown against Denv-2 (35%) (Capeding *et al.*, 2014). Developing and producing a vaccine

against dengue virus is the most challenging part because the vaccine have to be effective towards all the dengue serotypes (Normile, 2013). Additionally, there is a lot of aspects should be accounted for when developing and producing a vaccine such as the unstable current economic situation. Therefore, it is recommended to investigate the local plants that might lead to the development of the vaccine.

Dengue virus contains a single-stranded RNA of positive polarity, encoding for a single polyprotein precursor of 3,391 amino acids comprising three structural and seven non-structural (NS) proteins (Irie *et al.*, 1989). Dengue virus replication is dependent on the correct cleavage of this polyprotein and requires both host cell proteases and the virus-encoded, two-component protease, NS2B-NS3 (Falgout *et al.*, 1991; Yusof *et al.*, 2000). Inhibiting the NS2B-NS3 protease should prevent virus infection, thus, it serves as a target in the development of anti-viral drugs. Several studies have reported the use of natural products in ascertaining their inhibitory potentials against dengue NS2B-NS3 protease. Compounds isolated from the plant *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. (*Zingiberaceae* family) had shown inhibition against this protease (Kiat *et al.*, 2006; Othman *et al.*, 2008). Two cyclohexenyl chalcone derivatives namely, 4-hydroxypanduratin A and panduratin A, exhibited competitive inhibitions against DENV-2 NS2B-NS3 protease with reported inhibition constant, K_i values of 25 and 21 μM , respectively, while pinostrobin (flavonoid) exhibited non-competitive inhibition with IC_{50} value of 90 mg/mL. From computational investigations, it was suggested that pinostrobin bound into an allosteric binding site via interaction with Lys74, adjacent to one of the catalytic triad residues, Asp75, of the NS3 protein. (de Sousa *et al.*, 2015) had performed screening assays of flavonoids against NS2B-NS3 protease of DENV-2 and DENV-3. The results obtained showed that the flavonoids exhibited inhibitory activities with IC_{50} values ranging from 15 to 44 μM . Agathisflavone and myricetin turned out to be non-competitive

inhibitors with K_i values of 11 and 4.7 μM , respectively.

Malaysia is rich with many medicinal plants inspired from religious, traditional beliefs and practices. Amongst these plants, five plants were chosen to be the subject of this study, which are *Lawsonia inermis* (Inai), *Punica granatum* (Delima), *Dryobalanops aromatica* (Kapur barus), *Zizyphus jujuba* Lam. (Bidara) and *Zingiber zerumbet* (Halia Bara). All these plants are mentioned in Al-Quran and AHadith, and they are used and consumed in Malaysia as food and for various traditional medicinal purposes.

Lawsonia inermis, recognized as henna or inai was used as cosmetic purposes and highly demanded for the production of halal hair and nail dye products (Alia *et al.*, 1995; Badoni Semwal *et al.*, 2014; Dasgupta *et al.*, 2003). Besides that, it is traditionally used for treatment of inflammatory conditions, ringworm, dandruff, jaundice, calculus affliction, rheumatism, stomach disorder, skin diseases and sore throat (Alia *et al.*, 1995; Badoni Semwal *et al.*, 2014; Sultana *et al.*, 2009). *Lawsonia inermis* has been shown to possess numerous biological activities such as analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, anti-bacterial, anti-microbial, anti-fungal, anti-viral, anti-parasitic, anti-trypanosomal, anti-dermatophytic, anti-oxidant, anti-fertility, tuberculostatic and anti-cancer (Agarwal *et al.*, 2014; Muhammad, 2005). This plant was reported to contain lawsone, gallic acid, tannic acid, α -D-glucose, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, laxanthone (Nik *et al.*, 2012; Sultana *et al.*, 2009). It also contains carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes, flavone glycosides, pentacyclic triterpenes and fatty acids (Agarwal *et al.*, 2014; Badoni Semwal *et al.*, 2014).

Pomegranate or scientifically known as *Punica granatum* is distributed throughout Iran, Mediterranean countries, tropical

Africa, India, Malaysia and to some extent, in the United States (Viuda-Martos *et al.*, 2010; Zhang *et al.*, 2010). This plant was traditionally held sacred by several of the world's major religions such as Islam, Christian and Buddhist (Langley, 2000). In Malaysia, the young leaves of *Punica granatum* was burned and the ash is mixed with water and drank to treat stomach ache (Ong & Norzalina, 1999). A mixture of juice, pounded fruit peel, sugar and warm water is drank to treat vaginal white discharges. Ong *et al.* (2011) reported that the juice can also be used to treat leucorrhea, expel intestinal worms and to counter obesity for slimming purpose, the juice from the fruits is mixed with a little salt and drank regularly (Ong & Nordiana, 1999; Ong *et al.*, 2011). The extracts from different parts of this plant had been studied for numerous pharmacological activities, such as anti-oxidant, anti-tumor, anti-bacterial, astringent, anti-diarrheal and anti-obesity activities (Zhang *et al.*, 2010). Many researchers revealed that *Punica granatum* is a rich source of anthocyanins, polyphenols, tannins, phenolics, flavonoids, ellagitannins, proanthocyanidin and many minerals, including potassium, nitrogen, calcium, magnesium, phosphorus, and sodium (Heftmann *et al.*, 1966; Moneam *et al.*, 1988; Poyrazoğlu *et al.*, 2002; Viuda-Martos *et al.*, 2010).

Dryobalanops aromatica have been reported to yield terpenoid compounds like d-borneol, terpinen-4-ol, α -terpineol, α -pinene and caryophyllene, which are known to exhibit various activities such as anti-microbial, antiviral, anti-inflammatory and cytotoxic effects (Le *et al.*, 2016). Examples of compounds that have been isolated from this plant are malaysianol A, flexuosol A, vaticanol B, vaticanol C, laevifonol, ampelopsin E, α -viniferin, ϵ -viniferin, diptoindonesin A, bergenin, oleanolic acid acetate, hedragonic acid, dryobalanonoloic acid, methyl 11-oxoasiatate and dryobalanolide (Cheung & Wong, 1972; Wibowo *et al.*, 2011).

Zizyphus jujuba, a species from the Rhamnaceae family, is an indigenous plant possessing an array of medicinal properties,

attributed by a diverse group of secondary metabolites such as alkaloids, flavonoids, terpenoids and glucosides (Cheng *et al.*, 2000). *Zizyphus jujuba* has been used traditionally to treat insomnia, anxiety, sleep-related problems, hemorrhage and diarrhoea (Bencao, 1999; Chopra *et al.*, 1956; Lee, 1986; Sun *et al.*, 2011). Many researchers have reported multiple medicinal values of this plant such as anti-allergic, anti-ulcer, anti-fertility, wound healing and anti-oxidant properties (Ansari *et al.*, 2006; Chopda, 2009; Chopda & Mahajan, 2009; Ganachari & Kumar, 2004; Kim & Han, 1996; Su *et al.*, 2002). It is also found to be a potential natural pesticide in controlling *Aedes aegypti* mosquitoes (Devi & Bora, 2015).

Zingiber zerumbet is a species belonging to the Zingiberaceae family that has long been used in Asian popular medicine with many reported biological activities such as anti-cancer, anti-inflammation, anti-Alzheimer's disease, anti-viral, anti-tumor, anti-oxidant, anti-allergic, and anti-microbial activities (Abdul *et al.*, 2008; Abdul Hamid *et al.*, 2012; Matthes *et al.*, 1980; Singh *et al.*, 2012). The dichloromethane (DCM) and methanol (MeOH) extracts of the rhizome of *Zingiber zerumbet* demonstrated potent larvicidal activity against *Aedes nuneztovari* larvae and *Aedes aegypti* larvae, suggesting that *Zingiber zerumbet* could be an alternative to prevent the spread of mosquito vectors diseases (Bucker *et al.*, 2013). Examples of some compounds isolated from the essential oil of *Zingiber zerumbet* are zerumbone, zerumbone epoxide, humulene, zederone, camprene, diferuloylmethane, feruloyl-*p*-coumaroylmethane and di-*p*-coumaroylmethane.

In pursuing our interest in the studies of Malaysian medicinal plants (Abdul *et al.*, 2008; Ablat *et al.*, 2014; Awang *et al.*, 2010; Hamdi *et al.*, 2015; Othman *et al.*, 2006), this manuscript reports the inhibition of NS2B-NS3 serine protease (IC₅₀) by five traditional medicinal plants, namely *Lawsonia inermis* (Inai), *Punica granatum* (Delima), *Dryobalanops aromatica* (Kapur barus), *Zizyphus jujuba* Lam. (Bidara) and *Zingiber zerumbet* (Halia Bara).

MATERIALS AND METHODS

General procedures and plant materials

All plant materials were purchased from the local people and local traditional herbs suppliers. The voucher specimens were deposited at the Herbarium of the Department of Chemistry, Faculty of Science, University of Malaya. The industrial grade solvents were distilled prior to use for extraction.

Extraction

All plant materials were thoroughly rinsed with water. Each part of the plants (bark, leaves and rhizomes) were sliced and air-dried until constant mass. Dried plant materials were ground and the extraction process was carried out using maceration method, of which dried plant materials were first defatted with hexane and then soaked in ethyl acetate (EA) with periodical stirring for three days. Upon day 3, the EA extracts were separated from the plant materials through filtration. The filtrates were then dried using rotary evaporator to give dried extracts. To maximize extraction yield, the plant materials were subjected to the same extraction process for two more times. The dried extracts of each extraction were then combined to give the total EA extraction yield. After that, the same extraction process as above was carried out using methanol, MeOH (ethanol, EtOH for *Zingiber zerumbet*) and then water (except for *Zingiber zerumbet*) to give methanol, ethanol and water extracts, respectively.

Denv-2 protease inhibition assay

Protease inhibition study was performed using purified Denv-2 NS2B-NS3 protease (Abdul *et al.*, 2016; Nawi, 2015), and Boc-Gly-Arg-Arg-MCA as the substrate. The bioassay protocol was employed as described by Nawi (2015) and Abdul H.W. *et al.* (2016) with minor modification (Abdul *et al.*, 2016; Nawi, 2015). The enzyme concentration used in the assay was 0.5 μ M and substrate concentration was 10 mM in 200 mM Tris-HCl (buffer) with pH 8.5. The plant extract concentration used in this assay was 200 μ g/mL. All tests were performed in

quadruplicate. Firstly, a Tris buffer (pH 8) was pipetted to the wells, followed by 1 μ L of plant extract and 3.1 μ L of enzyme. Before adding the substrate, the enzyme and inhibitor were incubated at 37°C for 10 minutes. After adding the substrate, the reaction mixtures were incubated at 37°C for 60 minutes. All reactions were performed in 96-well plates with final volume of 100 μ L per well and fluorescence was detected using the Promega Glomax Multi Detection System microplate reader with excitation and emission wavelengths at 365 and 410-460 nm, respectively. For IC₅₀ determination, the same protocol was used as described before with serial dilutions of plant extract with concentration in the range of 0.78 to 200 μ g/mL.

RESULTS AND DISCUSSION

Twenty one plant extracts were screened for their inhibitory activity against Denv-2 NS2B-NS3 protease and the results are shown in Table 1. Thirteen extracts were found to be active at the concentration of 200 μ g/mL with percentage of inhibition more than 80%. The IC₅₀ values of these active extracts were determined via serial dilutions which gave IC₅₀ values ranging from 0.30 to 17.55 μ g/mL. The percentage inhibition of plant extracts against protease activity at different concentration (0.78 to 200.00 μ g/mL) was shown in Figures 1 and 2.

Two extracts were found to have 70% to 75% inhibition at 200 μ g/mL; while six extracts exhibited moderate to less inhibition (less than 65% inhibition). Extracts with more than 80% inhibition were subjected to IC₅₀ studies (except for MeOH extract of the barks of *Zizyphus jujuba*). The MeOH extract from the leaves of *Dryobalanops aromatica* showed the most potent activity (99.70 %). Moreover, the MeOH extracts of the barks of *Punica granatum* and *Lawsonia inermis* exhibited very strong inhibition as compared to their EA extracts. Interestingly, the barks of *Lawsonia inermis*, *Punica granatum* and *Zizyphus jujuba* as well as the leaves of *Dryobalanops aromatica* indicated an increase in inhibition against Denv-2 NS2B-NS3 protease with increasing polarity

Table 1. The percentage of inhibition of the crude extracts of *Lawsonia inermis*, *Punica granatum*, *Dryobalanops aromatica*, *Zizyphus jujuba* Lam. and *Zingiber zerumbet* against DENV2 NS2B/NS3 protease

Plants	Parts	Extracts	Mean percentage inhibition at 200 (µg/ mL)	IC ₅₀ ± Standard deviation (µg/ mL)
<i>Lawsonia inermis</i> (KL 5824)	Leaves (L)	Ethyl acetate	17.62	NT
		Methanol	56.52	NT
	Barks (B)	Ethyl acetate	86.43	0.77 ± 0.37
		Methanol	87.99	0.33 ± 0.14
<i>Punica granatum</i> (KL 5826)	Leaves (L)	Ethyl acetate	92.23	9.03 ± 3.21
		Methanol	91.02	3.10 ± 0.14
	Barks (B)	Ethyl acetate	85.36	17.55± 0.54
		Methanol	91.84	1.72 ± 0.29
<i>Dryobalanops aromatica</i> (KL 5829)	Leaves (L)	Ethyl acetate	95.10	3.49 ± 0.65
		Methanol	99.70	0.30 ± 0.16
	Barks (B)	Ethyl acetate	72.08	NT
		Methanol	96.52	0.51 ± 0.42
	Fruits (F)	Ethyl acetate	50.44	NT
		Methanol	75.00	NT
<i>Zizyphus jujuba</i> Lam. (KL 5828)	Leaves (L)	Ethyl acetate	94.44	7.83 ± 0.99
		Methanol	57.67	NT
	Barks (B)	Ethyl acetate	93.10	2.47 ± 0.72
		Methanol	94.58	NT
<i>Zingiber zerumbet</i> (KL 5835)	Rhizomes (R)	Hexane	63.25	NT
		Ethyl acetate	90.32	2.39 ± 0.86
		Ethanol	32.29	NT
Quercetin	Standard		100.00	3.17 ± 0.65

*NT: not tested

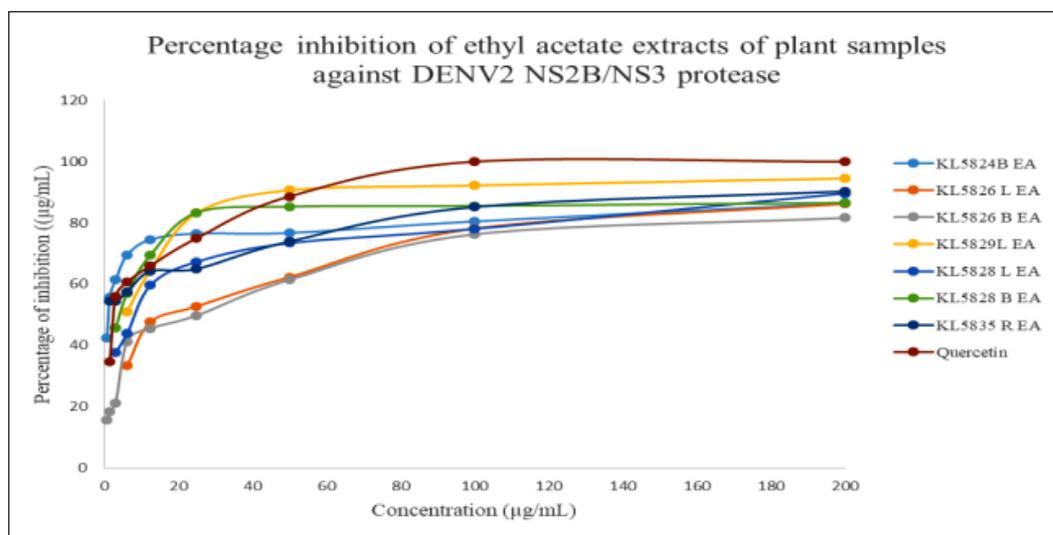


Figure 1. Percentage of inhibition of the ethyl acetate extracts of *Lawsonia inermis*, *Punica granatum*, *Dryobalanops aromatica*, *Zizyphus jujuba* Lam. and *Zingiber zerumbet* against DENV2 NS2B/NS3 protease at concentration ranging from 0.78 to 200.00 µg/mL.

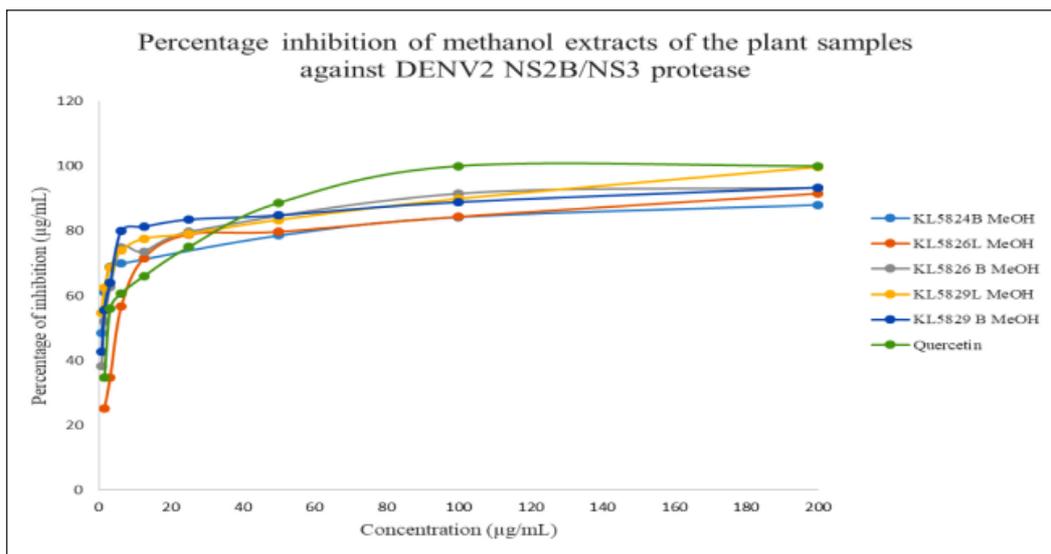


Figure 2. Percentage of inhibition of the methanol extracts of *Lawsonia inermis*, *Punica granatum*, *Dryobalanops aromatica*, *Zizyphus jujuba* Lam. and *Zingiber zerumbet* against DENV2 NS2B/NS3 protease at concentration ranging from 0.78 to 200.00 µg/mL.

of the solvent extracts (from EA to MeOH). This observation suggests that polar functionalities such as hydroxyl and carbonyl groups play an important role in facilitating interactions within the protease enzyme.

Among all the extracts, the MeOH extract of *Dryobalanops aromatica* showed the most potent inhibition against Denv-2 NS2B-NS3 protease with an IC_{50} value of 0.30 ± 0.16 µg/mL. In addition, leaves and bark of *Punica granatum* (MeOH extracts), barks of *Lawsonia inermis* (EA and MeOH extracts), *Dryobalanops aromatica* (MeOH extract), *Zizyphus jujuba* (EA extract) and the rhizomes of *Zingiber zerumbet* (EA extract) showed higher inhibiting potency as compared to quercetin (IC_{50} value of 3.17 µg/mL) which is used as a standard because it has demonstrated significant anti-dengue inhibitory activity (de Sousa *et al.*, 2015; Zandi *et al.*, 2011a; Zandi *et al.*, 2011b). Furthermore, Tan has reported the MeOH fraction of *Zingiber zerumbet* were potent against Denv2 NS2B/NS3 protease at 300 ppm with different extraction method (Tan *et al.*, 2006). However, the extracts of EA for the leaves of *Punica granatum*, *Dryobalanops aromatica*, *Zizyphus jujuba* and the bark of *Punica granatum* were found to be less

active. Based on the results obtained, it shows that the medicinal plants has potential to be investigated further for the development of anti-dengue agents.

CONCLUSIONS

The results of these preliminary studies showed that, in general, all the plants tested showed potent activity either in their leaves or bark extracts or both extracts. *Dryobalanops aromatica* (kapur barus), *Zizyphus jujuba* (bidara) and *Punica granatum* (delima) revealed potent activities in both leaves and bark extracts (more than 80% inhibition at 200 µg/mL). The MeOH extract of *Dryobalanops aromatica* leaves was found to exhibit very potent activity against Denv-2 NS2B-NS3 protease with an IC_{50} value of ten times lower than the standard quercetin. These medicinal plants have been reported to contain compounds such as apigenin (*Lawsonia inermis*), quercetin-3-*O*-rutinoside (*Zizyphus jujuba*), kaempferol-3-*O*-methylether (*Zingiber zerumbet*), quercetin (*Punica granatum*) and β -caryophyllene (*Dryobalanops aromatica*) (Chauhan & Chauhan, 2001; Guha

et al., 2011; Jang *et al.*, 2004; Le *et al.*, 2017; Wang *et al.*, 2014). The anti-protease activity of the extracts may be attributed to the presence of these compounds as several studies have shown that some flavonoids and bicyclic sesquiterpenes were active against dengue protease activity (Abd Kadir *et al.*, 2013; Kiat *et al.*, 2006; María *et al.*, 2018; Tang *et al.*, 2012).

Therefore, further studies such as bioassay-guided isolation of chemical constituents from the potent extracts especially for *Dryobalanops aromatica* can be carried out in the future. These extracts, along with pure compounds from the active extracts, can be tested for *in vitro* cell-based assays, such as plaque reduction or virus yield inhibition assays, to ascertain their anti-dengue properties in our efforts towards the development of anti-dengue therapeutics.

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REFERENCES

- Abd Kadir, S.L., Yaakob, H. & Mohamed Zulkifli, R. (2013). Potential anti-dengue medicinal plants: a review. *Journal of Natural Medicines* **67**(4): 677-689.
- Abdul, A.B., Abdelwahab, S.I., Al-Zubairi, A.S., Elhassan, M.M. & Murali, S.M. (2008). Anticancer and antimicrobial activities of zerumbone from the rhizomes of *Zingiber zerumbut*. *International Journal of Pharmacology* **4**(4): 301-304.
- Abdul Hamid, Z., Budin, S.B., Wen Jie, N., Hamid, A., Husain, K. & Mohamed, J. (2012). Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. *Journal of Zhejiang University Science B* **13**(3): 176-185.
- Abdul, H.W., Hariono, M., Tan, M.L. & Kamarulzaman, E.E.B. (2016). *Malaysia Patent No.:* Thioguanine derivatives: WO2016038562 A1.
- Ablat, A., Mohamad, J., Awang, K., Shilpi, J.A. & Arya, A. (2014). Evaluation of Antidiabetic and antioxidant properties of *Brucea javanica* seed. *The Scientific World Journal* **2014**: 8.
- Agarwal, P., Alok, S. & Verma, A. (2014). An update on Ayurvedic herb Henna (*Lawsonia inermis* L.): a review. *International Journal of Pharmaceutical Sciences and Research* **5**(2): 330.
- Alia, B., Bashir, A. & Tanira, M. (1995). Anti-inflammatory, antipyretic, and analgesic effects of *Lawsonia inermis* L.(henna) in rats. *Pharmacology* **51**(6): 356-363.
- Ansari, S., Bhatt, D., Masihuddin, M. & Khan, M. (2006). The wound healing and herbal drugs. *Herbal Drugs. Jay Pee Publication, New Delhi* 460-468.
- Awang, K., Nurul Azmi, M.N., In Lian Aun, L.I.L., Nazif Aziz, A.N., Ibrahim, H. & Hasima Nagoor, N. (2010). The apoptotic effect of 1'S-1'-acetoxychavicol acetate from *Alpinia conchigera* on human cancer cells. *Molecules* **15**(11): 8048-8059.
- Badoni Semwal, R., Semwal, D.K., Combrinck, S., Cartwright-Jones, C. & Viljoen, A. (2014). *Lawsonia inermis* L. (henna): Ethnobotanical, phytochemical and pharmacological aspects. *Journal of Ethnopharmacology* **155**(1): 80-103.
- Bencao, Z. (1999). *State Administration of Traditional Chinese Medicine (Vol. 5)*. Shanghai, China Shanghai Science and Technology Publishing Company.
- Bucker, A., Falcao-Bucker, N.C., Nunez, C.V., Pinheiro, C.C.D.S. & Tadei, W.P. (2013). Evaluation of larvicidal activity and brine shrimp toxicity of rhizome extracts of *Zingiber zerumbet* (L.) Smith. *Revista da Sociedade Brasileira de Medicina Tropical* **46**(3): 377-380.

- Capeding, M.R., Tran, N.H., Hadinegoro, S.R.S., Ismail, H.I., Chotpitayasunondh, T., Chua, M.N., Luong, C.Q., Rusmil, K., Wirawan, D.N., Nallusamy, R., Pitisuttithum, P., Thisyakorn, U., Yoon, I.K., van der Vliet, D., Langevin, E., Laot, T., Hutagalung, Y., Frago, C., Boaz, M., Wartel, T.A., Tornieporth, N.G., Saville, M. & Bouckenooghe, A. (2014). Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *The Lancet* **384**(9951): 1358-1365.
- Chauhan, D. & Chauhan, J. (2001). Flavonoid diglycoside from *Punica granatum*. *Pharmaceutical Biology* **39**(2): 155-157.
- Cheng, G., Bai, Y., Zhao, Y., Tao, J., Liu, Y., Tu, G., Ma, L., Liao, N. & Xu, X. (2000). Flavonoids from *Ziziphus jujuba* Mill var. *spinosa*. *Tetrahedron* **56**(45): 8915-8920.
- Cheung, H.T. & Wong, C.S. (1972). Structures of triterpenes from *Dryobalanops aromatica*. *Phytochemistry* **11**(5): 1771-1780.
- Chopda, M. (2009). *Studies on wound healing agents of plant origin*. Thesis, Faculty of Science, North Maharashtra University, Jalgaon, Maharashtra, India. 2009.
- Chopda, M. & Mahajan, R. (2009). Wound healing plants of Jalgaon district of Maharashtra state, India. *Ethnobotanical Leaflets* **2009**(1): 1.
- Chopra, R.N., Nayar, S.L. & Chopra, I.C. (1956). Glossary of Indian medicinal plants. *New Delhi: C SIR*.
- Dasgupta, T., Rao, A.R. & Yadava, P.K. (2003). Modulatory effect of Henna leaf (*Lawsonia inermis*) on drug metabolising phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice. *Molecular and Cellular Biochemistry* **245**(1): 11-22.
- de Sousa, L.R.F., Wu, H., Nebo, L., Fernandes, J.B., Kiefer, W., Kanitz, M., Bodem, J., Diederich, W.E., Schirmeister, T. & Vieira, P.C. (2015). Flavonoids as noncompetitive inhibitors of dengue virus NS2B-NS3 protease: Inhibition kinetics and docking studies. *Bioorganic and Medicinal Chemistry* **23**(3): 466-470.
- Devi, U. & Bora, D. (2015). Larvicidal efficacy of extracts of selected plants against *Aedes aegypti* (Linnaeus). *International Journal of Sciences and Applied Research* **2**(3): 89-94.
- Falgout, B., Pethel, M., Zhang, Y.-M. & Lai, C. (1991). Both nonstructural proteins NS2B and NS3 are required for the proteolytic processing of dengue virus nonstructural proteins. *Journal of Virology* **65**(5): 2467-2475.
- Ganachari, M. & Kumar, S. (2004). Anti-ulcer properties of *Ziziphus jujuba* Lam leaves extract in rats. *Journal of Natural Remedies* **4**(2): 103-108.
- Guha, G., Rajkumar, V., Kumar, R.A. & Mathew, L. (2011). Antioxidant activity of *Lawsonia inermis* extracts inhibits chromium (VI)-induced cellular and DNA toxicity. *Evidence-Based Complementary and Alternative Medicine* **2011**.
- Hamdi, O., Anouar, E., Shilpi, J., Trabolsy, Z., Zain, S., Zakaria, N., Zulkefeli, M., Weber, J.-F., Malek, S., Rahman, S. & Awang, K. (2015). A quantum chemical and statistical study of cytotoxic activity of compounds isolated from *Curcuma zedoaria*. *International Journal of Molecular Sciences* **16**(5): 9450.
- Heftmann, E., Ko, S.-T. & Bennett, R.D. (1966). Identification of estrone in pomegranate seeds. *Phytochemistry* **5**(6): 1337-1339.
- Irie, K., Mohan, P., Sasaguri, Y., Putnak, R. & Padmanabhan, R. (1989). Sequence analysis of cloned dengue virus type 2 genome (New Guinea-C strain). *Gene* **75**(2): 197-211.

- Jang, D.S., Han, A.R., Park, G., Jhon, G.J. & Seo, E.K. (2004). Flavonoids and aromatic compounds from the rhizomes of *Zingiber zerumbet*. *Archives of Pharmcal Reseach* **27**(4): 386-389.
- Kiat, T.S., Pippen, R., Yusof, R., Ibrahim, H., Khalid, N. & Rahman, N.A. (2006). Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. *Bioorganic and Medicinal Chemistry Letters* **16**(12): 3337-3340.
- Kim, H. & Han, S. (1996). *Zizyphus jujuba* and *Codonopsis pilosula* stimulate nitric oxide release in cultured endothelial cells and kidney tissues. *Asia Pacific Journal of Pharmacology* **11**(3-4): 121-128.
- Langley, P. (2000). Why a pomegranate? *BMJ: British Medical Journal* **321**(7269): 1153-1154.
- Le, T.-X., Ho, A. S.-H., Mah, S.-H., Wong, T.-W., Ong, H.-C., Loh, P. H.-M. & Lim, Y.-M. (2016). Determination of borneol and other chemical compounds of essential oil of *Dryobalanops aromatica* exudate from Malaysia. *Tropical Journal of Pharmaceutical Research* **15**(6): 1293-1297.
- Le, T.-X., Ho, A.S.-H., Mah, S.-H., Wong, T.-W., Ong, H.-C., Loh, P.H.-M. & Lim, Y.-M. (2017). Chemical composition of essential oil of exudates of *Dryobalanops aromatica*. *Tropical Journal of Pharmaceutical Research* **16**(3): 621-625.
- Lee, S.J. (1986). *Pen-Tsao-Kun-Mu* (Vol. 18). Beijing Chinese Book Store.
- María, C.F., Raquel, E.O. & Elena, E.S. (2018). Evaluation of *in vitro* antiviral activity of essential oil compounds against dengue virus. *Pharmacognosy Journal* **10**(1).
- Matthes, H.W.D., Luu, B. & Ourisson, G. (1980). Cytotoxic components of *Zingiber zerumbet*, *Curcuma zedoaria* and *Curcuma domestica*. *Phytochemistry* **19**(12): 2643-2650.
- Moneam, N.M.A., El Sharaky, A.S. & Badreldin, M.M. (1988). Oestrogen content of pomegranate seeds. *Journal of Chromatography A* **438**: 438-442.
- Muhammad, H.S.M.S. (2005). The use of *Lawsonia inermis* linn. (henna) in the management of burn wound infections. *African Journal of Biotechnology* **4**(9): 934-937.
- Nawi, M.S.M. (2015). *Ligand based drug discovery of novel dengue-2 NS2B-NS3 protease inhibitors*. Universiti Sains Malaysia.
- Nik, W.B.W., Zulkifli, F., Sulaiman, O., Samo, K.B. & Rosliza, R. (2012). Study of henna (*Lawsonia inermis*) as natural corrosion inhibitor for aluminum alloy in seawater. *IOP Conference Series: Materials Science and Engineering* **36**(1): 012043.
- Normile, D. (2013). Surprising new dengue virus throws a spanner in disease control efforts. *Science* **342**(6157): 415.
- Ong, H.C. & Nordiana, M. (1999). Malay ethno-medico botany in Machang, Kelantan, Malaysia. *Fitoterapia* **70**(5): 502-513.
- Ong, H.C. & Norzalina, J. (1999). Malay herbal medicine in Gemencheh, Negri Sembilan, Malaysia. *Fitoterapia* **70**(1): 10-14.
- Ong, H.C., Ruzalila, B.N. & Milow, P. (2011). Traditional knowledge of medicinal plants among the Malay villagers in Kampung Tanjung Sabtu, Terengganu, Malaysia. *Indian Journal of Traditional Knowledge* **10**(3): 460-465.
- Othman, R., Ibrahim, H., Mohd, M.A., Mustafa, M.R. & Awang, K. (2006). Bioassay-guided isolation of a vasorelaxant active compound from *Kaempferia galanga* L. *Phytomedicine* **13**(1-2): 61-66.
- Othman, R., Kiat, T.S., Khalid, N., Yusof, R., Irene Newhouse, E., Newhouse, J.S., Alam, M. & Rahman, N.A. (2008). Docking of noncompetitive inhibitors into dengue virus type 2 protease: understanding the interactions with allosteric binding sites. *Journal of Chemical Information and Modeling* **48**(8): 1582-1591.

- Poyrazoğlu, E., Gökmen, V. & Arték, N. (2002). Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *Journal of Food Composition and Analysis* **15**(5): 567-575.
- Singh, C.B., Nongalleima, K., Brojendrosingh, S., Ningombam, S., Lokendrajit, N. & Singh, L.W. (2012). Biological and chemical properties of *Zingiber zerumbet* Smith: a review. *Phytochemistry Reviews* **11**(1): 113-125.
- Su, B.N., Cuendet, M., Farnsworth, N.R., Fong, H.H., Pezzuto, J.M. & Kinghorn, A.D. (2002). Activity-guided fractionation of the seeds of *Ziziphus jujuba* using a cyclooxygenase-2 inhibitory assay. *Planta Medica* **68**(12): 1125-1128.
- Sultana, N., Choudhary, M.I. & Khan, A. (2009). Protein glycation inhibitory activities of *Lawsonia inermis* and its active principles. *Journal of Enzyme Inhibition and Medicinal Chemistry* **24**(1): 257-261.
- Sun, Y.-F., Liang, Z.-S., Shan, C.-J., Viernstein, H. & Unger, F. (2011). Comprehensive evaluation of natural antioxidants and antioxidant potentials in *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou fruits based on geographical origin by TOPSIS method. *Food Chemistry* **124**(4): 1612-1619.
- Tan, S.K., Pippen, R., Yusof, R., Rahman, N.A., Ibrahim, H. & Khalid, N. (2006). Screening of selected Zingiberaceae extracts for dengue-2 virus protease inhibitory activities. *Sunway Academic Journal* **3**: 1-7.
- Tang, L.I.C., Ling, A.P.K., Koh, R.Y., Chye, S.M. & Voon, K.G.L. (2012). Screening of anti-dengue activity in methanolic extracts of medicinal plants. *BMC Complementary and Alternative Medicine* **12**: 3.
- Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Pomegranate and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety* **9**(6): 635-654.
- Wang, S., Zhang, J., Zhang, Z., Gao, W., Yan, Y., Li, X. & Liu, C. (2014). Identification of chemical constituents in the extract and rat serum from *Ziziphus jujuba* mill by HPLC-PDA-ESI-MSn. *Iranian Journal of Pharmaceutical Research* **13**(3): 1055.
- Wibowo, A., Ahmat, N., Hamzah, A.S., Sufian, A.S., Ismail, N.H., Ahmad, R., Jaafar, F.M. & Takayama, H. (2011). Malaysianol A, a new trimer resveratrol oligomer from the stem bark of *Dryobalanops aromatica*. *Fitoterapia* **82**(4): 676-681.
- World Health Organization. (2017a). Dengue and severe dengue [Press release]. Retrieved from <http://www.who.int/mediacentre/factsheets/fs117/en/>.
- World Health Organization. (2017b). Update on the dengue situation in the Western Pacific region. Dengue Situation Update Number **507**: 1-5.
- Yusof, R., Clum, S., Wetzel, M., Murthy, H.K. & Padmanabhan, R. (2000). Purified NS2B/NS3 serine protease of dengue virus type 2 exhibits cofactor NS2B dependence for cleavage of substrates with dibasic amino acids *in vitro*. *Journal of Biological Chemistry* **275**(14): 9963-9969.
- Zandi, K., Teoh, B.T., Sam, S.S., Wong, P.F., Mustafa, M.R. & AbuBakar, S. (2011a). Antiviral activity of four types of bioflavonoid against dengue virus type-2. *Virology Journal* **8**: 560-560.
- Zandi, K., Teoh, B.T., Sam, S.S., Wong, P.F., Mustafa, M.R. & AbuBakar, S. (2011b). *In vitro* antiviral activity of fisetin, rutin and naringenin against dengue virus type-2. *Journal of Medical Plants Research* **5**.
- Zhang, L., Gao, Y., Zhang, Y., Liu, J. & Yu, J. (2010). Changes in bioactive compounds and antioxidant activities in pomegranate leaves. *Scientia Horticulturae* **123**(4): 543-546.