UNIVERSITI PUTRA MALAYSIA

A SYSTEMATIC REVIEW ON HERBAL PLANTS FROM SOUTHEAST ASIA REPORTED TO POSSESS ANTI-DENGUE ACTIVITY

BY

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A SYSTEMATIC REVIEW ON HERBAL PLANTS FROM SOUTHEAST ASIA REPORTED TO POSSESS ANTI-DENGUE ACTIVITY

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A PROJECT PAPER SUBMITTED AS PARTIAL REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE (BIOMEDICAL SCIENCES)

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ABSTRACT

A Systematic Review on Herbal Plants from Southeast Asia Reported to Possess Anti-Dengue Activity

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Introduction: Dengue is one of the alarming diseases in Southeast Asian countries. The use of modern symptomatic medicines to eradicate them was found to be less efficient in overcoming increasing dengue cases. **Objective**: This systematic review was aimed to provide information such as the name of herbal plants, parts of herbal plants, active compounds present, type of extracts, assays and study design used, IC50/CC50/ LC50 values obtained from the herbal plants with anti-dengue properties studies in Southeast Asia and assess the risk of bias of the selected studies using low, medium and high risk. **Methodology**: This systematic review was done based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The data was collected from 11 eligible articles obtained from the electronic search (PubMed, Science Direct, Scopus and Wiley) from the year 2016–2021. The data were then analyzed using descriptive statistical methods in the Microsoft Excel Version 2013. **Results**: Thailand and Malaysia are the most prominent countries involved in anti-dengue herbal plants research followed by Vietnam and Indonesia. Some of the herbal plants from Genus Cinnamomum studied mostly among the selected articles on potential vector control agents. Most of the studies used leaf parts due to its medicinal properties. Flavonoids are active compounds commonly found in examined herbal plants as they possess significant anti-dengue properties. Most of the studies have been conducted from ethanol and methanol extract as compared to aqueous extract as it has the potential to yield high extraction of active compounds present in the leaf parts. Denv-2 protease inhibition assay, foci-forming unit assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay were the assays used in in-vitro studies and larvicidal bioassay was the assay used in the in-vivo studies. IC50 and CC50 values are measured in in-vitro studies and LC50 values are measured in in-vivo studies. **Discussion**: Some of the herbal plant species from Genus Cinnamomum possess anti-dengue activity for potential vector control agents. The leaf part of herbal plants using ethanol/methanol extract could be one of the suggestions to produce anti-dengue remedy and vector control agents. Moreover, it is recommended to choose herbal plants that contain flavonoids in high proportion. The type of assay used and the values of IC50/CC50/ LC50 measured will be based on the study design used. The percentage score for risk of bias assessment shows all the eligible 11 articles have low risk of biases. **Conclusion**: This review has provided some information on herbal plants in Southeast Asia with potential anti-dengue properties.

Keywords: Dengue, Southeast Asia, Herbal plants, Systematic review

ABSTRAK

Kajian Sistematik terhadap Tumbuhan Herba dari Asia Tenggara Dilaporkan Memiliki Kegiatan Anti-Denggi

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Pengenalan: Denggi adalah salah satu penyakit yang membimbangkan di negara-negara Asia Tenggara. Penggunaan ubat simptomatik moden untuk membasmi mereka didapati kurang berkesan dalam mengatasi peningkatan kes denggi. **Objektif**: Kajian sistematik ini bertujuan untuk memberikan maklumat seperti nama tanaman herba, bahagian tanaman herba, sebatian aktif, jenis ekstrak, ujian dan reka bentuk kajian yang digunakan, nilai IC50 / CC50 / LC50 yang diperoleh daripada tanaman herba di Asia Tenggara dan menilai risiko bias kajian terpilih menggunakan risiko rendah, sederhana dan tinggi. **Metodologi**: Kajian sistematik ini dilakukan berdasarkan garis panduan Item Pilihan untuk Ulasan Sistematik dan Meta-Analisis (PRISMA). Data dikumpulkan dari 11 artikel yang memenuhi syarat yang diperoleh dari carian elektronik (PubMed, Science Direct, Scopus dan Wiley) dari tahun 2016-2021. Data kemudian dianalisis menggunakan kaedah statistik deskriptif dalam Microsoft Excel Versi 2013. **Hasil**: Thailand dan Malaysia adalah negara paling terkenal yang terlibat dalam penyelidikan tanaman herba anti-denggi diikuti oleh Vietnam dan Indonesia. Sebilangan tanaman herba dari Genus Cinnamomum banyak dikaji di antara artikel terpilih. Sebilangan besar kajian menggunakan bahagian daun kerana sifat perubatannya. Flavonoid adalah sebatian aktif yang biasa dijumpai pada tanaman herba yang diperiksa kerana mempunyai sifat anti-denggi yang signifikan. Sebilangan besar kajian telah dilakukan dari ekstrak etanol dan metanol dibandingkan dengan ekstrak berair kerana berpotensi menghasilkan ekstraksi sebatian aktif yang tinggi pada bahagian daun. Uji penghambatan protease Denv-2, pengujian unit pembentuk fokus dan ujian 3- (4,5-dimetilthiazol-2-yl) -2,5-diphenyl-2H-tetrazolium bromida (MTT) adalah ujian yang digunakan dalam kajian in-vitro dan bioassay larvicidal adalah ujian dalam kajian in-vivo. Nilai IC50 dan CC50 diukur dalam kajian in-vitro dan nilai LC50 diukur dalam kajian in-vivo. **Perbincangan**: Sebilangan spesies tumbuhan herba dari Genus Cinnamomum mempunyai aktiviti anti-denggi untuk menjadikan agen kawalan vektor yang berpotensi. Bahagian daun tumbuhan herba yang menggunakan ekstrak etanol / metanol dapat menjadi salah satu cadangan untuk menghasilkan ubat anti-denggi dan agen kawalan vektor. Lebih-lebih lagi, disarankan untuk memilih tanaman herba yang mengandungi flavonoid dalam jumlah yang tinggi. Jenis ujian yang digunakan dan nilai IC50 / CC50 / LC50 yang diukur akan berdasarkan reka bentuk kajian. Skor peratusan untuk penilaian risiko berat sebelah menunjukkan semua 11 artikel yang layak mempunyai risiko berat sebelah yang rendah. **Kesimpulan**: Ulasan ini telah memberikan maklumat mengenai tanaman herba di Asia Tenggara yang berpotensi untuk membasmi jangkitan denggi.

Kata kunci: Denggi, Asia Tenggara, Tumbuhan herba, Kajian sistematik

APPROVAL

It is hereby certified that I have read this project paper entitled “A Systematic Review on Herbal Plants from Southeast Asia Reported to Possess Anti-Dengue Activity” by LINESH KUMAR A/L SELVARAJA and in my opinion, it is satisfactory in term of scope, quality, and presentation as a fulfilment of the requirement for the course of SBP4990.

_____

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Dengue is an infection from the bite of Aedes mosquitoes which includes Ae.aegypti and Ae.albopictus. The incubation period of the disease ranges from three to fourteen days after infection. The symptoms of the disease include high fever, muscle pain, headache and vomiting. According to WHO, the number of dengue cases in Southeast Asian countries has been rising dramatically in recent years (Dengue and Severe Dengue, 2021). This is because the tropical season of Southeast Asian countries favors the breeding of Aedes species which will eventually give rise to dengue cases (Ooi & Gubler, 2009). The potential for a dengue transmission includes a number of mosquitoes, a number of susceptible humans, suitable climate and virus transmission potential.

The temperature plays a vital role in the transmission of the dengue and influences the dynamic modelling of vector–host interaction (Polwiang, 2015). Dengue transmission occurs best at temperature of 28.7 °C. In relation to that, the rainfall countries from the Southeast Asian region provide the best breeding sites for the mosquitoes to hatch and further develop into the adult stage which give rise to the number of dengue mosquitoes and infections. Currently, there are only symptomatic medications available for dengue viral disease. These include oral fluid administration and antipyretic treatment with acetaminophen are given to dengue patients (Rajapakse et al., 2012). Unfortunately, these medications

give some side effects such as excessive acetaminophen intake could lead to liver injury (Yoon et. al., 2016). On the other hand, Dengvaxia which was a vaccine initially given to people to reduce the number of dengue cases has been found to increased risk of severe dengue illness for people who had never had the disease, if they contracted the virus after being vaccinated (Thomas & Yoon, 2019).

Currently, there is research going on about finding alternative medications for dengue. According to research from Oyebode et al. (2016), 80 percent of the African and Asian population depend on herbal medicine as their primary health care. One of the regions where numerous anti-dengue studies on herbal plants are being carried out is the Southeast Asian region due to its high biodiversity. For instance, *Myristica fatua and Acorus calamus* are two medicinal herbs found in Indonesia that are thought to have anti-dengue properties (Rosmalena et al., 2019). Moreover, *Andrographis paniculata* is a plant found in tropical Southeast Asian countries found to be inhibitors of dengue virus (Kaushik et al., 2021). Not only that, conventional Chinese herbal plants such as *Morus alba* and *Dryopteris crassirhizoma* have anti-dengue properties (Maryam et al., 2020).The inefficiency and side effects of current anti-dengue drugs encourage the need for anti-dengue drugs from herbal plants. Plant-based medicines are typically thought to be safer and also less damaging and toxic than synthetic drugs (Abd Kadir et al., 2013). Despite various scattered scientific reports on herbal plants in the Southeast Asian region with potential to attenuate dengue infection, no attempt has been made to systematically review these findings to validate all reports and summarize on the most effective herbal plants with anti-dengue effect based on some of the

important data presented such as name of the plant, origin of collection, parts of plant used, active compound present, type of extract, study design, LC50/IC50/CC50 values obtained and the outcome of the research.

In brief, it is important to find a solution for dengue infection and herbal plants would be one of the options. In order to extend our research on herbal plants, this systematic review would give us ideas on potential herbal plants from Southeast Asia with anti-dengue properties.

1.2 Problem statement

There is a huge rise in dengue cases around the world. According to Leo (2012), a total of 390 million dengue virus infections per year with 96 million presenting clinically (with any severity of disease). Another study on the incidence of dengue fever, 3.9 billion individuals are at danger of contracting the virus (Simo et al., 2019). Despite the fact that there is a risk of infection in 129 nations, with Asia accounting for 70% of the total burden (Brady et al., 2012). In 2020, dengue cases continue to rise in Brazil, Bangladesh, Brazil, Nepal, Indonesia, India, Thailand, and Timor-Leste and countries such as Bangladesh, Malaysia, Vietnam and Philippines recorded 101,000, 131,000, 320,000 and 420,000 cases respectively.

The rise in the number of cases urged the scientist to invent new medicines as the current anti-dengue drugs/treatment were found to be less efficient. This is because current medications are basically symptomatic medication which includes Acetaminophen, Panadol, fluid replacement therapy which will not be efficient to overcome the burden of the disease. Due to these issues, scientists have shifted their focus towards the potential of herbal plants as a potential source of new anti-dengue agent(s) to fight Aedes species infection. This is because natural products have emerged as the primary testing ground for antiviral medications based on traditional medicinal methods. Traditional medicines are based on practice, experience and knowledge based on indigenous people's cultural beliefs to diagnose, treat and prevent diseases. Antiviral activity has been found in traditional medicinal herbs and has been utilized to treat viral infections

(Betancur-Galvis et al., 1999). There is now ample research done on herbal plants with possible anti-dengue properties around the world. One of the regions where numerous anti-dengue studies on herbal plants are being carried out is the Southeast Asian region due to its high biodiversity.

As a result, this review was conducted to determine the herbal plants from Southeast Asia for effective production of novel anti-dengue drugs and vector control agents from herbal plants.

In summary, there are three questions addressed in this present systematic review:

1. What are the names of herbal plants, parts of herbal plants and active compounds present in the herbal plants with anti-dengue properties?

2. What are the type of extracts, assays and study design used and IC50/CC50/ LC50 values obtained from the herbal plants with anti-dengue properties studies?

3. What is the level of risk of biasness access from the articles obtained from the systematic review?

1.3 Significance of the study

Even though many researchers have studied possible ways to treat dengue infection through herbal plants, there is a lack of systematic review done regarding the possible herbal based to aid in effective production of novel anti-dengue drugs and vector control agents to overcome increasing dengue cases (Saleh & Kamisah, 2020; Rather et al., 2017). A systematic review study is essential to be conducted as it will identify, evaluate, and summarize the findings of all relevant individual studies on herbal plants from Southeast Asia that have the potential to invent new medicine and possible vector control agents from herbal plants. Thus, this study will systematically review all the possible medications and vector control agents from plant sources in Southeast Asia for dengue infection and organize them in an organized way. In relation to that, conducting a systematic review will aid in the production of precise and reliable findings, the delivery of essential information to researchers, the improvement of consistency and generalizability of the results, and the overall precision of the results (Ganeshkumar & Gopalakrishnan, 2013).

1.4 Limitation

There are several limitations of the study that have been identified and should be corrected in the future study. First, the present study only includes herbal plants from Southeast Asian countries with anti-dengue properties. This means the result of the study might be limited to herbal plants in Southeast Asian countries, not all over the world, even though many herbal plants around the world have the potential to eradicate dengue. Next, the present study is only limited from the articles published from the year 2016-2021. This is because the present study is aimed to include only the research that has been done in the recent years.

On the other hand, the present study only includes articles that have been published and does not include any books. Even though some of the books highlighted Southeast Asian herbal plants with anti-dengue properties, according to new research (Ganeshkumar & Gopalakrishnan, 2013), a systematic review is a process of gathering and analyzing all journals on a particular topic and it should only include research journals. Thus, books removed from the present study.

Finally, the total number of databases included in the present study is only four even though more databases are available for the finding of research articles. This is because the analysis of research articles consumes more time and present study conducted only for a short period of time. So it is better to limit the number of databases for article searching in order to complete the present study in the given time frame.

1.5 Research objectives

General objective

To provide information associated with effective production of novel anti-dengue drugs and vector control agents from herbal plants.

Specific objectives

1. To identify the name of herbal plants, parts of herbal plants and active compounds present in the herbal plants with anti-dengue properties.

2. To identify the type of extracts, assays and study design used and IC50/CC50/ LC50 values from the herbal plants with anti-dengue properties research papers.

3. To assess risk of bias using low, medium and high risk.

1.6 Hypothesis

It is hypothesized that the information on the herbal plants from Southeast Asian countries for effective production of novel anti-dengue drugs and vector control agents has been identified.

1.7 Conceptual framework

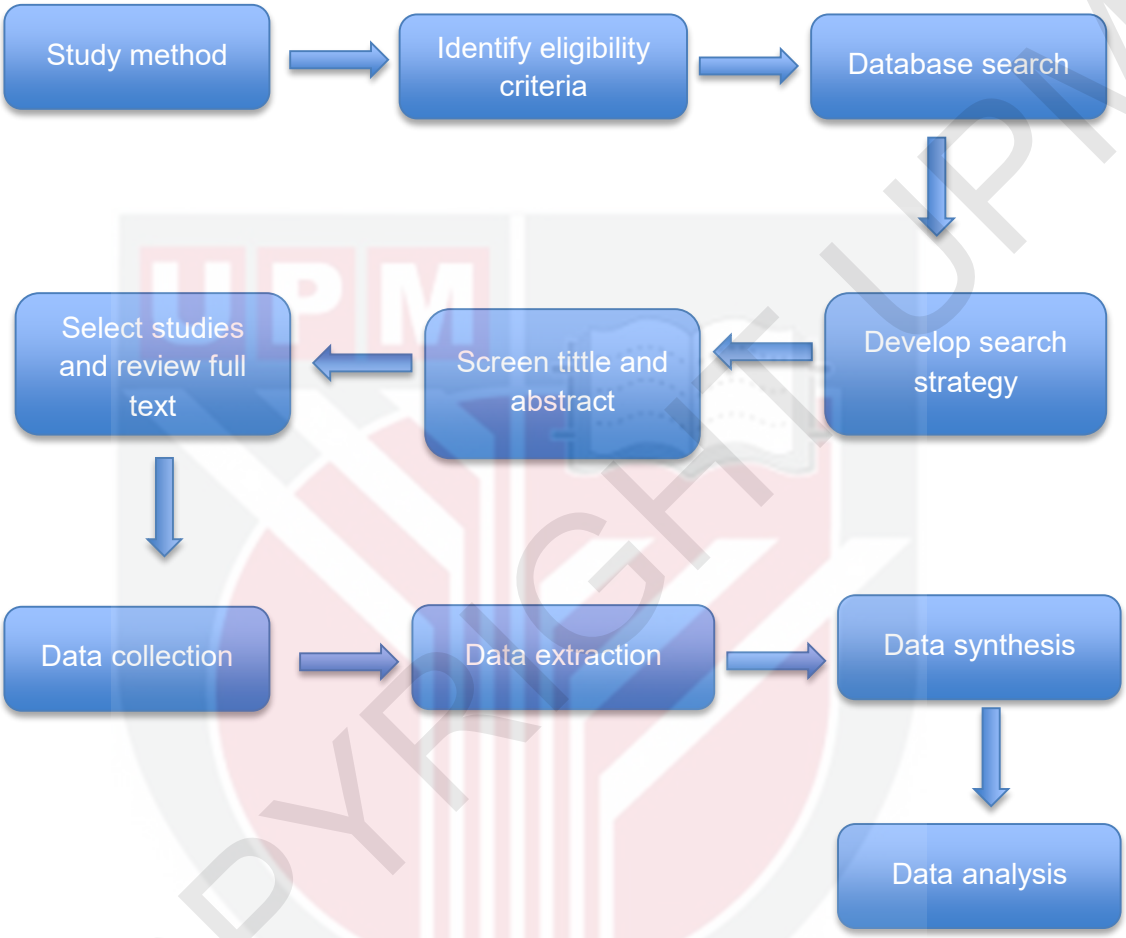

Figure 1.7 Conceptual Framework

1.8 Definition of terminology

Dengue- is a tropical disease caused by the dengue virus from Aedes mosquitoes.

Systematic review- uses systematic methods to identify, select and critically appraise all relevant research, and to gather and analyze data from the studies that are included in the review.

Southeast Asia- is the part of Asia, consisting of countries such as Cambodia, Myanmar, Timor-Leste, Laos, Indonesia, Malaysia, Singapore, Philippines, Thailand and Vietnam.

Herbal plants- plants that possess therapeutic properties or exert beneficial pharmacological effects on the human or animal body.

IC50 - “Inhibitory Concentration” which means the amount of the chemical in the air that kills 50% of the test animals.

LC50 - “Lethal Concentration” which means the chemical concentration in air that kills 50% of the test animals during the observation period.

CC50 - “Cytotoxic Concentration” which means the amount of the chemical that reduced the cell viability by 50%.

Active compound- a small molecule that specifically inhibits, stimulates or otherwise alters the production or activity of a target.

Study design- is a set of methods and procedures used to gather and analyze data on variables specified in a particular research area.

Assay- is an analytic procedure used in laboratories to qualitatively assess or quantitatively measure the presence, amount, or functional activity of a target substance.

CHAPTER 2

LITERATURE REVIEW

2.1 Dengue

According to the Centers for Disease Control and Prevention (CDC), dengue fever is a tropical disease which spreads from the bite of an *Aedes* species (*Ae. aegypti* or *Ae. albopictus*) mosquito. Dengue is a disease in more than 90 countries around the world. Every year around 300 million people get dengue infection and 80 million people get sick due to the infection, and 20,000 of them pass away from severe dengue infection. The symptoms of dengue viral infection includes fever, rash, aches, nausea and vomiting in mild dengue whereas restlessness, belly pain, vomiting blood and bleeding from nose in severe dengue. The incubation period of dengue viral infection will be from 2-7 days. Some of the key facts of dengue viral infection is that 1 out of 4 of the infected people will get sick, the symptoms of dengue viral infection can be mild to severe. Moreover, severe dengue viral infection can be dangerous sometimes whereby an infected person requires care at a hospital within a few hours of infection. Currently there are no specific medications for dengue and we depend solely on symptomatic medications to treat dengue.

2.2 Prevalence of dengue

Before 1970, severe dengue epidemics only occurred in 8 to 9 countries but now the disease is endemic with more than 100 countries seriously affected, in Asia representing approximately 70% of the global burden of disease. In relation to that, in Southeast Asia the number of dengue cases has been increasing drastically due to the tropical season of Southeast Asian countries that favors the breeding of Aedes species (Ooi & Gubler, 2009). Following the first epidemic in 1954 in the Philippines, epidemics have occurred in nearly all countries in Southeast Asia and are a huge concern among the Southeast Asian population.

2.3 Current medications of dengue infection

There are no specific medications for dengue across the world. The current medication given to dengue infected patients is symptomatic medications which primarily targets to reduce the symptoms of dengue such as pain, fever and muscle aches. The options are paracetamol and acetaminophen. In addition, the first vaccine for dengue was known as Dengvaxia which was developed by Sanofi Pasteur. It was first given approval to be used in more than 10 countries around the world. However, some safety concerns had arrived and it was then withdrawn from use by the people.

2.3.1 Side effects of current medications

Even though symptomatic medications are useful in overcoming dengue cases but in some situations, it leads to unhealthy conditions such as excessive acetaminophen intake could lead to liver injury (Yoon et. al., 2016). Moreover, it is found that Dengvaxia use has been linked to severe dengue infection in those who had never had the disease before and developed it after being vaccinated (Thomas & Yoon, 2019).

2.4 Plant based medications for dengue infection

Herbal plants from Indonesia such as *Myristica fatua and Acorus calamus* are found to have anti-dengue properties (Rosmalena et al., 2019). Moreover, *Andrographis paniculata,* which is a plant found in tropical Southeast Asian countries, is found to be an inhibitor of dengue virus (Kaushik et al., 2021). Not only that, members from genus Cinnamomum from Vietnam have shown promise as potential vector control agents (Dai et al., 2020).

2.4.1 Efficiency of plant based medications

Plants are commonly used in treatment and prevention of specific ailments and diseases (Sofowora et al., 2013. This is because plant-based medicines are generally considered safer as well as less harmful and toxic than synthetic drugs (Abd Kadir et al., 2013). In a research from Kasole et al. (2019), traditional medicines such as indigenous vegetables like avocado seed, lemon grass and hare lettuce leaves found to be an effective way in managing diabetes mellitus.

As for dengue, extracts from plants such as Cissampelos pareira, *Azadirachta indica*, Hippophae rhamnoides and *Carica papaya* were found to be effective in direct inhibitory effect and showed improvement in clinical symptoms of dengue virus (P. K. Singh & Rawat, 2017). In their research, it is also mentioned that the clinical trial of *C. papaya* showed an increase in platelet count and recovery in a quick period. So, these plants might need to be explored further as probable candidates for drug discovery against dengue.

CHAPTER 3

METHODOLOGY

3.1 Study method

The method to conduct the systematic review based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines.

3.2 Eligibility criteria

SPIDER guidelines (Cooke et al., 2012) was used as a framework to construct eligibility criteria in qualitative systematic review study.

3.2.1 Sample

The sample would be herbal plants from Southeast Asian countries.

3.2.2 Phenomenon of Interest

The study will focus on herbal plants with anti-dengue properties.

3.2.3 Design

All experimental studies that involve in vitro and in vivo studies.

3.2.4 Evaluation

Studies that prove the herbal plants in Southeast Asia that can overcome dengue are based on name of herbal plants, parts of herbal plants used, active compound

present, type of extract used, type of assay used, study design used, the values of IC50, LC50 and CC50 obtained from studies conducted.

3.2.5 Research type

All qualitative, quantitative and mixed-methods research were included in the study.

3.3 Database search

The literature studies for the study were searched from different databases such as Scopus, Wiley, ScienceDirect and PubMed.

3.4 Develop search strategy

To search for literature studies few search strategies were developed by using keywords such as (herbs, anti-dengue properties and Southeast Asian countries), Boolean operators such as (AND, OR, NOT), truncation (*) and filters such as (Time frame: 2006 to 2021, Full text). The search limits for the systematic review includes (i) journal articles in English language, (ii) availability of full text articles and (iii) articles published within the last 5 years range. Few search strategies was tried but due to low number articles obtained it was removed and the finalized search strategy used was herbs AND dengue AND Malaysia, herbs AND dengue AND Vietnam, herbs AND dengue AND Brunei, herbs AND dengue AND Myanmar, herbs AND dengue AND Cambodia, herbs AND dengue AND Timor-Leste, herbs AND dengue AND Singapore, herbs AND dengue AND Indonesia,

herbs AND dengue AND Laos, herbs AND dengue AND Thailand and herbs AND dengue AND Philippines.

3.5 Article selection

The articles that met the eligibility criteria will be included into selected articles for data extraction and synthesis later. It would be conducted in three phases which are identification, screening and inclusion of selected articles.

3.5.1 Article identification

The articles will be identified based on the search strategy as mentioned above in the search engines by two independent reviewers.

3.5.2 Article screening

The article screening will be done in two phases. In the first phase, the articles will be screened based on title and abstract. All the irrelevant articles and duplicates will be removed. In the second phase, the articles will be screened based on full text articles obtained. Articles that do not meet the eligibility criteria will be removed.

3.5.3 Inclusion of selected articles

The articles that meet eligibility criteria will be included for the data extraction and synthesis process later.

3.6 Data collection

The selected articles will be exported into Mendeley which help to organize the research findings in an organized way.

3.7 Data extraction

The data that will be extracted from the finalized articles includes title of article, the published year of the article, the name of country the plant collected, parts of plant used to create the extract, the active compound found in the plant, the type of extract used, the assay and study design used to conduct the research, the values of IC50/LD50/CC50 obtained from the research and outcome of the research. All these data will be transferred to Microsoft Excel spreadsheet.

3.8 Data synthesis

The data extracted will be used to make tables containing information on the place of collection of the plant, the plant name, the part of the plant, the active compound present, the type of extract used, the assay and the study design used in the study, IC50/LC50/CC50 values obtained from the study and outcome of the study.

3.9 Data analysis

The data analysis will be done using the Microsoft Excel Version 2013. The analyzed data is presented in the form of tables, clustered column graph, pie charts and stacked bar graph using descriptive statistical methods.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Search results

From the articles searching in the databases a total of 865 records were revealed in which 196 were duplicates. The end total of 669 records were examined. Further 157 records were excluded because they were books. The full text of the remaining 512 were recovered to decide whether to be included in the review. Another 501 were excluded for not meeting the inclusion criteria. The reasons for the exclusion criteria were: other than dengue related studies, articles from non-Southeast Asian countries and review articles. Data from 11 included studies were extracted and presented in the Table 4.1 and Table 4.2.

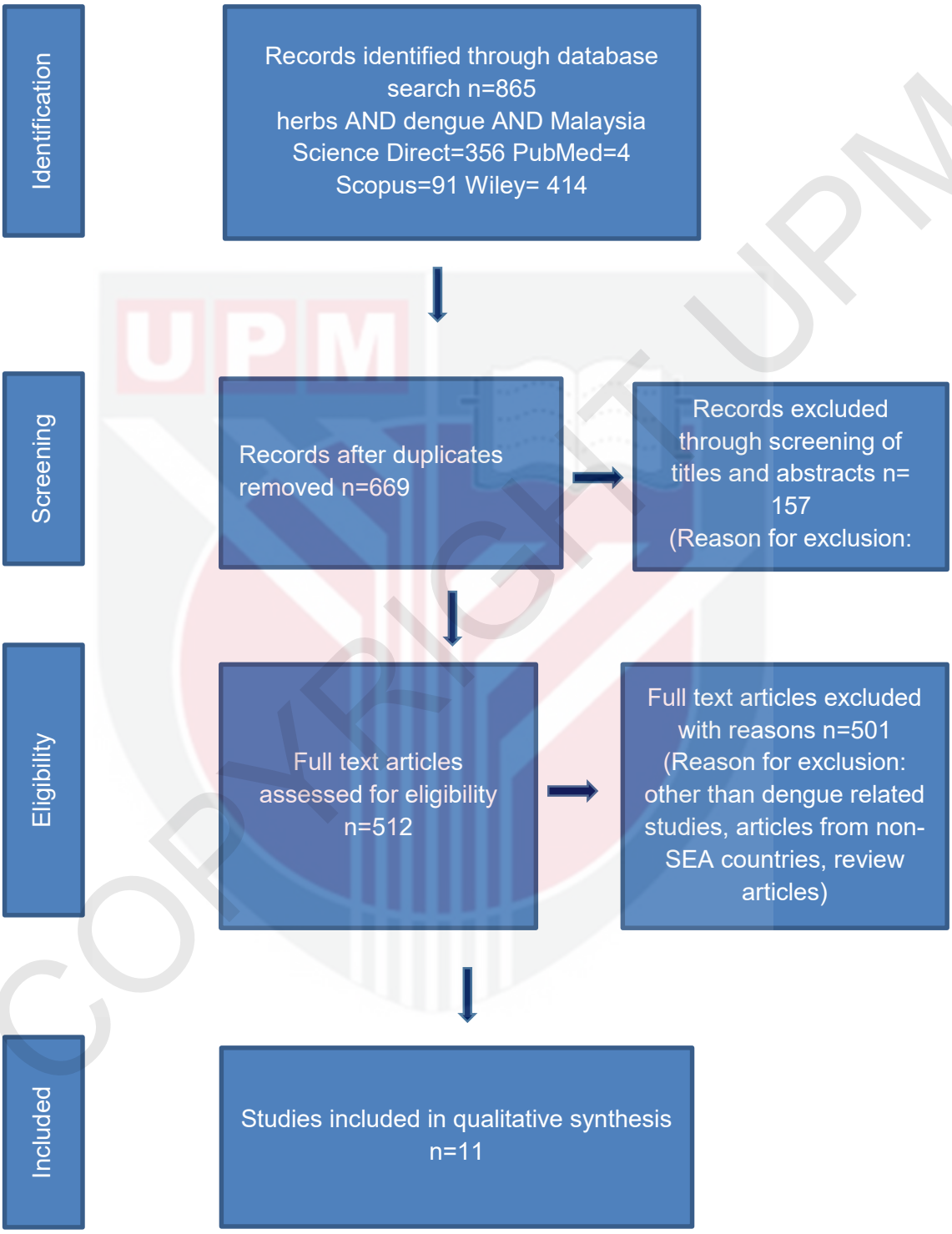

Figure 4.1: Flow chart of the study selection process

4.2 Description of the included studies

From the included 11 studies, information on the place of collection of the plant, the plant name, the part of the plant and the type of extract used in the study were summarized in the table 4.1 and the active compound present, assay and study design used in the study, IC50/LC50/CC50 values obtained from the study and outcome of the study were summarized in the table 4.2.

Table 4.1 The name of the country, name of plant, parts of plant used and type of extract used.

Country	Plant name	Part of plant	Type of extract	Reference & Study focus
Malaysia	Houttuynia cordata	Leave	Ethanol extract	K. Chiow et al. (2016) & Possible anti-dengue drug development
Thailand	Petroselinum crispum (Umbelliferea e)	Fruit	Ethanol extract	Intirach et al. (2016) & Possible vector control agent
Malaysia	Syzygium aromaticum, Cymbopogon citratus and Cinnamon cassia	Bark	Methanol extract	Sekar and Rahim (2017) & Possible vector control agent
Thailand	Zanthoxylum limonella Alston (Rutaceae)	Fruit	Ethanol extract	Soonwera and Phasomkusol sil (2017) & Possible vector control agent
Thailand	Acacia pennata	Shoot tips	Ethanol extract	Thongwat et al. (2017) & Possible

				vector control agent
Thailand	Acacia Catechu	Leave	Aqueous extract	Panya et al. (2018) & Possible anti-dengue drug development
Malaysia	Dryobalanops aromatica	Leave	Methanol extract	Salleh et al. (2019) & Possible anti-dengue drug development
Indonesia	Acorus calamus, Cymbopogon citratus, and Myristica fatua	Leave	Methanol extract	Rosmalena et al. (2019) & Possible anti-dengue drug development
Malaysia	Dryopteris crassirhizoma , Morus alba	Rhizome	Aqueous extract	MARYAM et al. (2020) & Possible anti-dengue drug development
Thailand	Cassia, Cinnamon, East Indian lemongrass, Bay, Sweet basil, Holy basil, and Ginger	Leave	Methanol extract	Tanawat et al. (2020) & Possible vector control agent

Vietnam	Cinnamomum	Leave	Aqueous extract	Dai et al. (2020) & Possible vector control agent

Table 4.2 Active compound present in the plant, assay used, study design, IC50/LC50/CC50 values obtained and outcome/summary of the research.

Active compound	Assay used	Study design	IC50/LC50/CC50 values	Outcome/ Summary	Reference & Study focus
Flavonoids	MTT assay	In vitro	IC50 value is 20.83 µg/mL	H. cordata has much potential for the development of antiviral agents against dengue infections	K. Chiow et al. (2016) & Possible anti-dengue drug development
Thymol	Larvicidal bioassay	In vivo	LC50 values are 43.22, 44.50, and 44.03 ppm	Anti-Mosquito activity of P. crispum oil against A. aegypti can be an alternative supplement in mosquito management	Intirach et al. (2016) & Possible vector control agent
Isoprenoids	-	In vivo	-	Repellent activity with mean of 66.67% after three consecutive tests	Sekar and Rahim (2017) & Possible vector control agent

Limonoids	Larvicidal bioassay	In vivo	LC50 value range from 54–58 ppm	Z. limonella oil can be used as an effective adulticide, larvicide and oviposition deterrent against Ae. aegypti	Soonwera and Phasomkusolsil (2017) & Possible vector control agent
Pinitol	Larvicidal bioassay	In vivo	LC50 value range from 39.45-50.75 µg/mL	It will be an effective mosquito larvicidal and pupicidal from A. pennata extracts	Thongwat et al. (2017) & Possible vector control agent
Carotenoids	Foci-forming unit assay	In vitro	IC50 value is 0.18 µg/ml	Extract from Acacia catechu demonstrated the most promising anti-DENV activity	Panya et al. (2018) & Possible anti-dengue drug development
Flavonoids	Denv-2 protease inhibition assay	In vitro	IC50 value range from 0.30-17.55 µg/mL	Dryobalanops aromatica (kapur barus),Zizyphus jujuba (bidara) and Punica granatum (delima) revealed potent activities in the	Salleh et al. (2019) & Possible anti-dengue drug development

				extracts (more than 80% inhibition at 200 µg/mL)	
Flavonoids	MTT assay	In vitro	CC50 value of A. calamus, C. citratus, and M.fatua extracts were 424.93 g/mL, 183.74 g/mL and 474.42 g/mL respectively	Methanolic extract of A. calamus, C. citratus, and M. fatua have antiviral effect to DENV without any cytotoxic effect	Rosmalena et al. (2019) & Possible anti-dengue drug development
Flavonoids	Foci-forming unit assay	In vitro	IC50 value of D. crassirhizoma is 130 µg/mL and M. alba is 221 µg/mL	Dryopteris crassirhizoma and Morus alba extracts have the equal potential against all DENV serotypes	MARYAM et al. (2020) & Possible anti-dengue drug development
Phenylpropanoid	Larvicidal bioassay	In vivo	LC50 value is 0.03ppm	All seven commercial essential herb oils have high efficacy against Aedes aegypti	Tanawat et al. (2020) & Possible vector control agent

Eugenol	Larvicidal bioassay	In vivo	LC50 value is 17.4 µg/mL	Cinnamomum has effective larvicidal activity against Ae. aegypti and Ae. albopictus	Dai et al. (2020) & Possible vector control agent

4.3 Dengue research in last five years

According to the above column bar graph, we can notice that in the last five years, Thailand, Malaysia, Indonesia and Vietnam are the four Southeast Asian countries involved in the research of herbal plants with potential anti-dengue activity. Thailand is the most prominent country that is involved in the anti-dengue study from herbal plant's extract with a total of five and Malaysia is the next country after Thailand with a total of four studies followed by Vietnam and Indonesia each one study respectively. This could be attributed to Thailand's huge rural population, which makes extensive use of traditional herbal plants in their daily lives (Phumthum & Balslev, 2018). In addition to that, Thailand is also the home of many ethnic groups whom depend on herbal plants for their health problems. This urges them to find potential herbal plants with anti-dengue activity through their extensive research. On the other hand, the number of dengue cases has been alarming in Malaysia. Until mid-year of 2020, Malaysia had recorded 50,988 dengue cases and 88 dengue fatalities nationwide (Ying, 2020). This had led them to find some alternative medication for dengue viral infection through research on herbal plants.

Figure 4.2 The number of research on herbal plants with potential anti-dengue activity done by Southeast Asian countries in the year 2016-2021.

4.4 Common herbal plants studied

The most common herbal plants investigated for the possible vector control agents were from the Genus Cinnamomum and all the herbal plants were reported highly effective at killing Aedes mosquito larvae (Sekar and Rahim 2017; Tanawat et al., 2020; Dai et al., 2020).

Table 4.3: Table highlighting the commonly studied herbal plants from the selected studies.

Genus	Species	Reference
Cinnamomum (8)	Cinnamomum cassia (2)	Sekar and Rahim (2017), Tanawat et al. (2020)
	Cinnamomum zeylanicum (1)	Tanawat et al. (2020)
	Cinnamomum damhaensis (1)	Dai et al. (2020)
	Cinnamomum longipetiolatum (1)	Dai et al. (2020)
	Cinnamomum ovatum (1)	Dai et al. (2020)
	Cinnamomum polyadelphum (1)	Dai et al. (2020)
	Cinnamomum tonkinense (1)	Dai et al. (2020)

4.5 Parts of the herbal plants

Among the various parts of the herbal plants used in medical research, usually the root (25.74%) is the common parts used in the research, followed by leaves parts (24.26%) (Abadi & Shimels, 2018). In the case of analyzing anti-dengue potential of herbal plants, most of the selected studies (6 out of 11) used leave part of the plant to make the plant extract. According to the United States Department of Agriculture, the leaf part of a plant contains the most medicinal properties compared to other parts of the plant (Medicinal Botany - Plant Parts Used, 2019). This could be a reason most of the researchers select leaves for their research on anti-dengue. Moreover, leaves parts of herbal plants were the most frequently used in the preparation of ethnomedicinal recipes (Aziz et al., 2018).

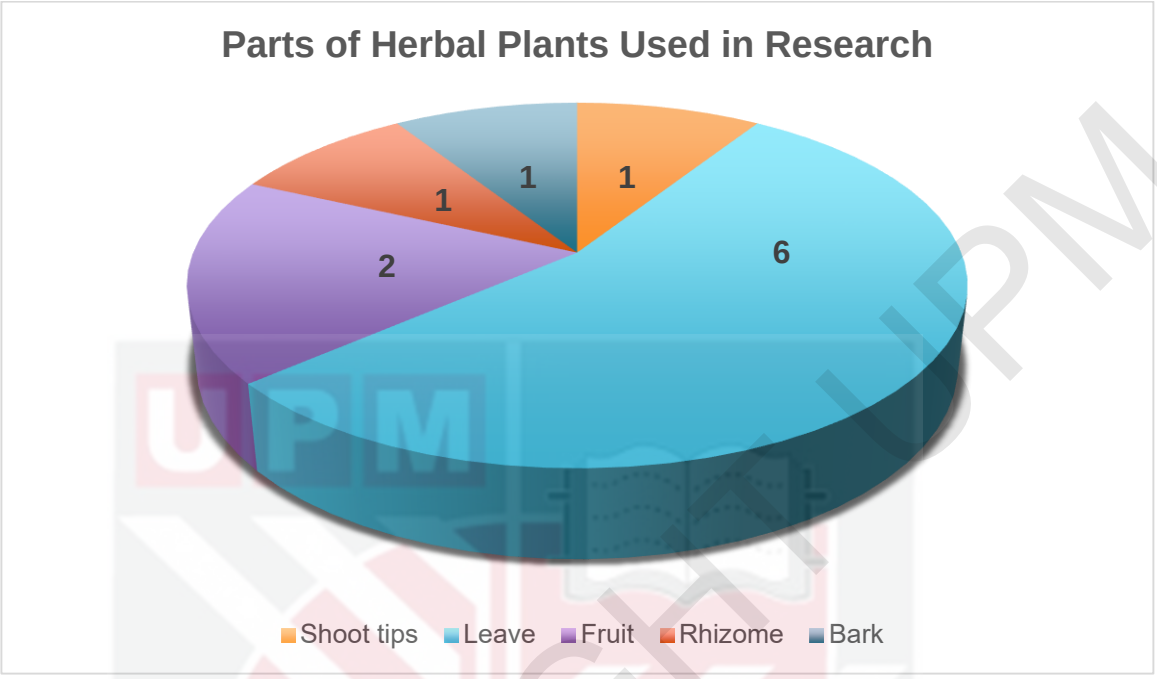

Figure 4.3 Parts of the herbal plants that are used in the selected studies.

4.6 Solvents of the Extract

Solvents such as acetone, ethanol, methanol and water are used for extracting bioactive compounds from the plant material. The above stacked bar graph shows that among the selected studies, most of the studies have been conducted from ethanol and methanol extract as compared to aqueous extract. In a study from Truong et al. (2019), methanol is suggested as the best solvent for obtaining large levels of phytochemical components in plants. Moreover, methanol extraction yields high content of flavonoid and phenolic compounds compared to ethanol extract (Iloki-Assanga et al., 2015). Not only that, a study from Do et al. (2014) revealed that methanol extract yields more active compounds from the plants compared to other extracts such as acetone. These

can be an indication for the future researchers to use ethanol and methanol in their plant related research.

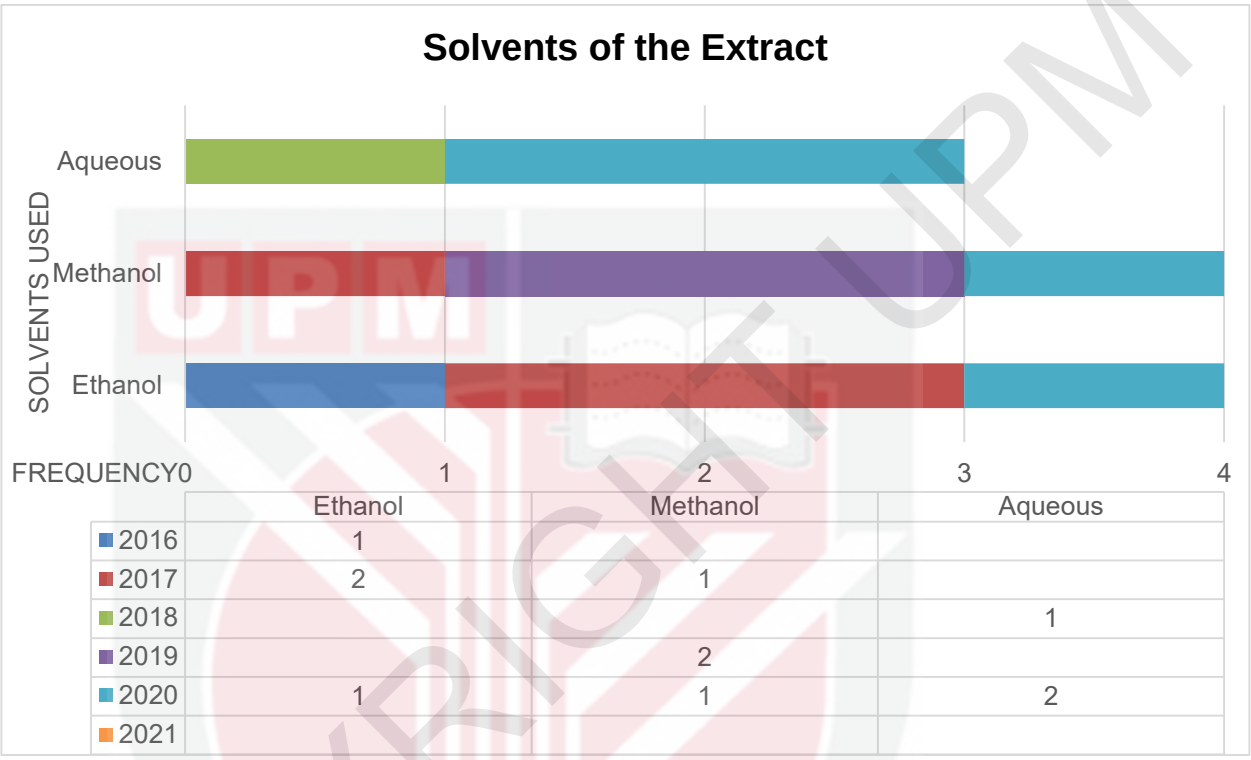

Figure 4.4 Solvents of extracts that were used in the selected studies.

4.7 Active compound

Bioactive substances are phytochemicals with therapeutic potential and the ability to alter metabolic activities, resulting in positive effects (Carbonell-Capella et al., 2014). There are different types of bioactive compounds that usually can be identified in plants. The examples include carotenoids, thymol, flavonoids, carnitine, choline, coenzyme Q, eugenol, dithiolethiones, phytosterols, limonoids, phytoestrogens, glucosinolates, pinitol, polyphenols, and taurine. From the pie chart above, 4 in the 11 included studies, flavonoids are the most found in

the examined herbal plants for anti-dengue drug development. This gives us indication to further our research on herbal plants related to flavonoids to create novel anti-dengue drugs from herbal plants to reduce and overcome dengue viral infection.

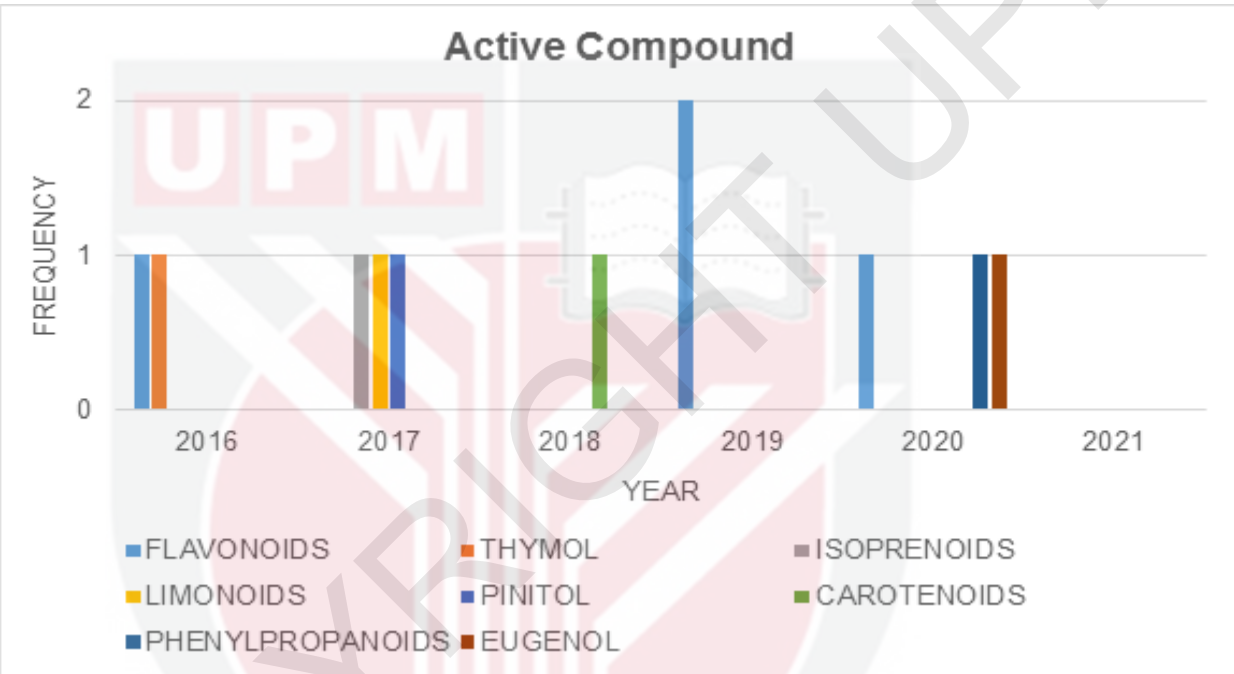

Figure 4.5 Active compounds that are commonly found in the selected studies.

4.8 Types of Assays

Larvicidal bioassay was used to conduct the research for potential vector control agents compared to other assays. Bioassays became the preference because it can detect very small concentrations of test substances and it is more reliable than chemical substances as it is done on living organisms (Advani, 2019). In this case, the test substance would be the extract of herbal plants with potential anti-dengue activity. On the other hand, MTT (3-(4,5-dimethylthiazol-2-

yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay was used in the anti-dengue drug development studies. This could be owing to the simplicity and speed with which the tests were completed, the consistency of the data, and the clinical correlation observed between in vitro and in vivo testing (Liu et al., 2020). But there are several disadvantages of MTT assay such as the less sensitivity of detection method for detecting viable cell number and the cytotoxic effects exhibited by the MTT reagents. Not only that, adding the reagent to estimate cell viability can actually be damaging to the cells or even can kill the cells during an experiment (Riss, 2021). Foci forming unit assay (an immunostaining technique) and DenV-2 protease inhibition assay are also used in the anti-dengue drug development studies whereby it can detect infectious virus particles before a plaque is formed. It has excellent detection of infectious virus in clinical samples but it is high in human labor (Grigorov et al., 2011).

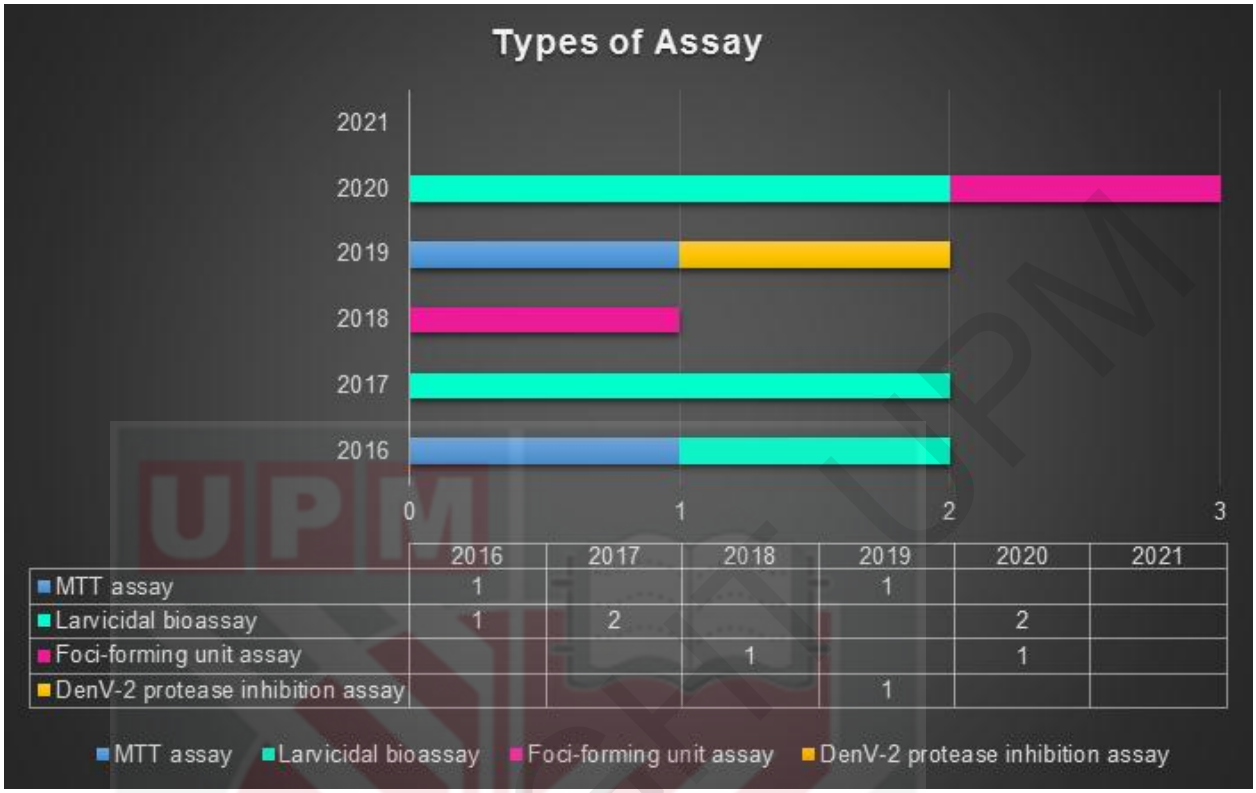

Figure 4.6 Types of assays that were used in the selected studies.

4.9 Study design and IC_{50}/LC_{50}/CC_{50} values

LC_{50} values can be measured in in-vivo studies whereas CC_{50} or IC_{50} values can be measured in in-vitro studies. Half-maximal inhibitory concentration (IC_{50}) quantifies the amount of medicine necessary to half-inhibit a biological process and identifies the level of potency of an antagonist drug in medical research (Aykul & Martinez-Hackert, 2016). On the other hand, the LC_{50} value is the concentration of a test substance that kills 50% of the animals. In this case usually the lethal effects of a test compound will be tested from breathing a chemical. The chemical normally will be a gas or vapor first mixed in a known concentration in a special air chamber where the animals to be tested will be

placed (L. Smith, 1984). This concentration will be usually quoted as parts per million (ppm) or milligrams per cubic meter (mg/m^3). Moreover, the 50% cytotoxic concentration (CC_{50}) was described as the concentration that reduced 50% of the cell viability (Du et al., 2018).

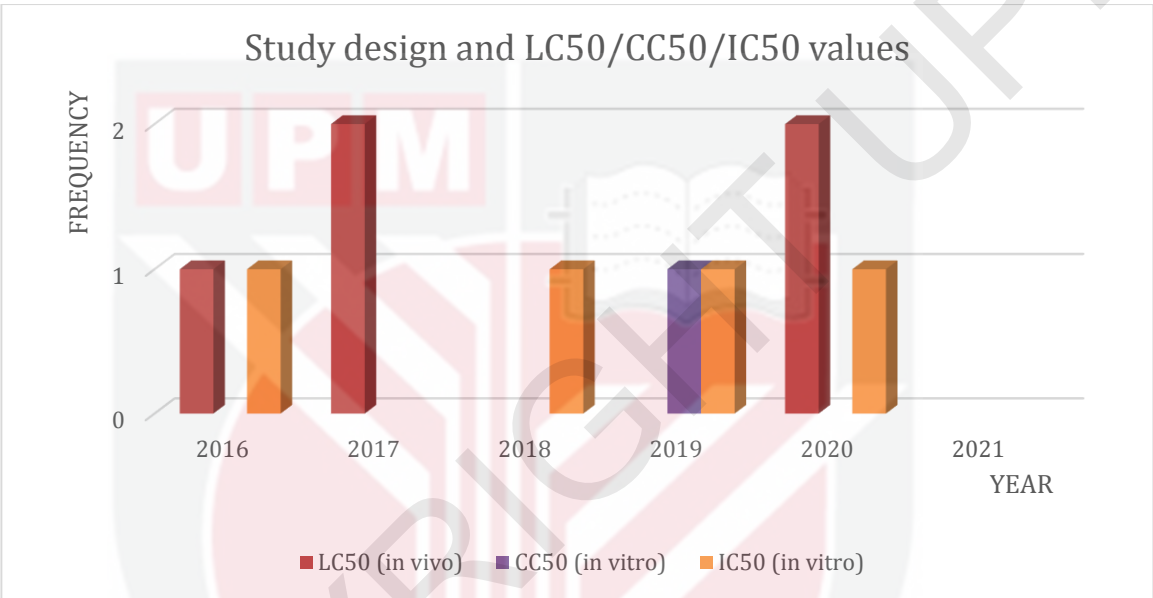

Figure 4.7 IC_{50}/LC_{50}/CC_{50} values obtained from the selected studies.

4.10 Risk of bias assessment

The risk of bias assessment was done on the selected 11 articles by incorporating 11 criteria to establish quality of the evidence. Scores given based on the degree to which the specific criteria were met (“yes” = 2, “partial” = 1, “no” = 0) and the criteria not applicable to a particular study design were marked “n/a” (Kmet, Lee, and Cook, 2004). A percentage score was then calculated for each paper by summing the total score obtained across relevant items and dividing by the total possible score (Example: score obtained/22 x 100). The selected articles

are categorized as high quality if the percentage obtained is higher than 50% and as low quality if the percentage obtained is lower than 50%. The selected 11 articles have the percentage of more than 50% indicating all the selected papers are high quality papers with low level risk of biases. The outcome of the risk of bias assessment done on the selected studies presented on table 4.4 below.

Table 4.4: The outcome of the risk of bias assessment

Article	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Percentage Score
Maryam et al. (2020)	2	2	2	2	1	2	2	2	2	2	2	95.45
Dai et al. (2020)	2	1	1	2	2	1	1	1	2	2	2	77.27
Salleh et al. (2019)	2	2	2	2	2	1	2	2	2	2	2	95.45
Sekar and Rahim (2017)	2	1	2	2	2	1	1	1	2	2	2	81.82
Rosmalena et al. (2019)	2	2	2	1	2	2	2	2	2	2	2	95.45
Tanawat et al. (2020)	2	1	2	1	2	1	2	2	2	2	2	86.36
Intirach et al. (2016)	2	2	2	2	2	1	2	1	2	2	2	90.91
Thongwat et al. (2017)	2	2	2	1	2	2	1	1	2	2	2	86.36
K. Chiow et al. (2016)	2	2	2	2	2	2	2	2	2	2	2	100
Soonwera and Phasomkusolsil (2017)	2	2	2	2	2	1	2	2	2	2	2	95.45
Panya et al. (2018)	2	2	2	2	2	2	2	1	2	2	2	95.45

Item 1 Question/Objective is sufficiently described
Item 2 Study design is evident and appropriate
Item 3 Method is well described and appropriate
Item 4 Subject is sufficiently explained and described
Item 5 Outcome is clearly explained
Item 6 Sample size is appropriate
Item 7 Analysis is well described and conducted appropriately
Item 8 Estimate of variance is reported for main results
Item 9 Controlled for confounding
Item 10 Result is reported in sufficient data
Item 11 Result support the conclusion

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, this review has provided information on some herbal plants in Southeast Asia with potential to eradicate dengue (6 out of 11 studies on possible vector control agents and 5 out of 11 studies on possible anti-dengue drug development). Moreover, this review has given a platform for future scientists to further explore the development and production of vector control agents and anti-dengue drugs from natural resources. Data from this systematic review could be useful in the conservation of Southeast Asian herbal plants biodiversity with potential anti-dengue properties for the future use.

Herbal plants are found abundantly in various parts of Southeast Asian countries. Thus, it is recommended to conduct more research on the potential of those herbal plants as alternative medications to overcome dengue infection. Moreover, it is advisable to extend its scope of location to all the countries around the world to get more journals for systematic review to get more information about the herbal plants with anti-dengue properties. This will help prevent high rates of dengue infection and mortality rate among the Southeast Asian population.

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