Chapter 6

Secondary Metabolite Research in Malaysia: Current Status and Future Prospects

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Abstract

Herbal medicine is gaining acceptance worldwide for their effective pharmacological effects and relative safety. Plants have metabolic pathways that lead to the production and accumulation of secondary metabolites such as alkaloids, glycosides, and terpenoids that exhibit various biological activities. Plant secondary metabolites have been the major foci of investigation for several years and have been successfully used against a number of communicable and noncommunicable diseases such as diabetes, cancer, and viral infections. This chapter will explore Malaysian plants, their secondary metabolites, and their biological/medicinal properties with a particular focus on some selected species under a national project. Other aspects such as plant tissue culture to produce secondary metabolites and a case study on the use of secondary metabolites in the prevention and treatment of dengue fever are also described. While a lot of effort has been put in, further research and development into plant secondary metabolites are needed including using the plant tissue culture approach toward reaching high-value herbal industry.

Keywords: biological activities, dengue, medicinal plants, secondary metabolites, tissue culture

1. Introduction

Secondary metabolites are defined as natural products that often have an ecological role in regulating the interactions between plants and their environment [1]. Plants execute several defense

mechanisms against pathogenic microbes, herbivores, and diseases. One of these defense mechanisms is the accumulation of secondary metabolites such as alkaloids, flavonoids, glycosides, terpenes, saponins, and tannins, which are important in health, food, and environmental fields [2]. For centuries, herbal extracts from a variety of plant species have been used as remedies for a wide spectrum of diseases. Majority of these extracts, the medicinal properties of which are attributable to the secondary metabolites present in the plants and serve as lead molecules in current drug design and development. It is interesting to note that a large number of drugs that have been approbated within the last 25 years are of natural origin and incorporate bioactive material with "drug-like properties" [3]. Even more pertinent is the fact that 12 of the world's 25 best-selling pharmaceutical agents were obtained from natural products [4].

Classic examples of plant secondary metabolites that later become lead for drug development include artemisinin from *Artemisia annua* (sweet wormwood, qinghao) for treatment of malaria, digoxin from *Digitalis lanata* (foxglove) for treatment of various heart conditions, and paclitaxel from *Taxus brevifolia* (Pacific yew) as chemotherapy medication.

This chapter will explore Malaysian plants, their secondary metabolites, and their medicinal properties with a particular focus on some selected species under a national project. Other aspects such as plant tissue culture to produce secondary metabolites and a case study on the use of secondary metabolites in the treatment of dengue fever are also described.

2. Secondary metabolites from Malaysian plants

A recent review by Buenz et al. described the ethnopharmacologic contribution to bioprospecting natural products [5]. Many databases of traditional medicine uses of natural products have been established, for instance, the PharmDB-K (covering traditional Korean medicine) [6], FERN Ethnomedical Plant Database (covering fern species) [7], AfroDb (covering African medicinal plants) [8], and Traditional Chinese Medicine Information Database (TCM-ID) [9]. In 2002, the Institute for Medical Research (IMR), Malaysia, was granted an approval to host a global electronic information resource on traditional and complementary medicine (TCM) known as Glob*in*Med (http://www.globinmed.com). The project was initially discussed during the 12th Commonwealth Health Ministers Meeting in Barbados in 1998 where the main idea was to establish a working group on TCM-related activities. Glob*in*Med also partners with ASEAN Task Force on Traditional Medicine (ATFTM) and several local institutions to enhance its service.

As one of the 17 megadiversity countries with 15, 000 estimated known plant species, Malaysia has a great potential for bioprospecting toward discovery of compounds with medicinal value. Together with ethnopharmacologic evidences from its rich traditional medicinal practice, several plant species have been selected for the agriculture NKEA-EPP1 (National Key Economic Area, Entry Point Project 1: High Value Herbal Products) [10], namely, tongkat ali (*Eurycoma longifolia* Jack), Misai Kucing (*Orthosiphon aristatus* (Blume) Miq.), Hempedu Bumi (*Andrographis paniculata* (Burm.f.) Nees), Dukung Anak (*Phyllanthus niruri* L.), Kacip Fatimah (*Marantodes pumilum* (Blume) Kuntze (*syn. Labisia pumila* (Blume) Mez)), Mengkudu (*Morinda citrifolia* L.), Roselle (*Hibiscus sabdariffa* L.), ginger (*Zingiber officinale*), Mas Cotek (*Ficus deltoidea* Jack), Belalai

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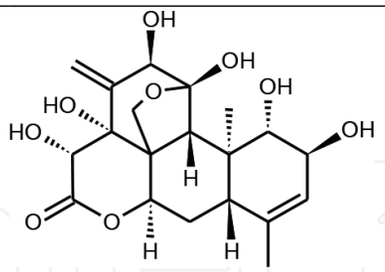
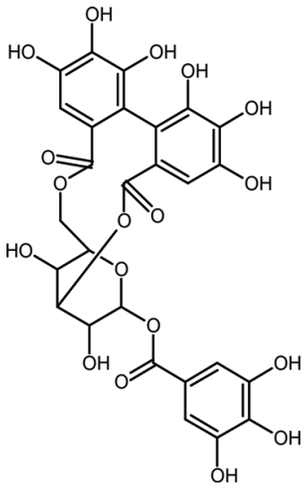
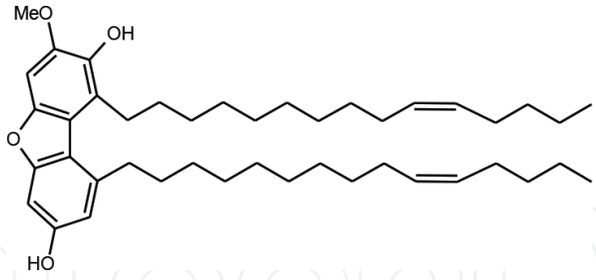
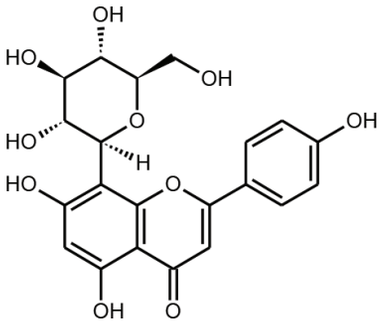
Plant	Secondary metabolites	Biological activity	Reference
Eurycoma longifolia **Jack (Tongkat Ali)**	 Eurycomanol (class: quassinoids)	Antimalarial against *P. falciparum*	[12]
Phyllanthus niruri L. **(Dukung Anak)**	 Corilagin (class: tannins)	Anti-hyperalgesic	[13]
Marantodes pumilum (Blume) Kuntze (syn. Labisia pumila (Blume) Mez **(Kacip Fatimah)**	 Fatimahol (class: alkylphenols)	Not available	[14–16]
Ficus deltoidea **Jack (Mas Cotek)**	 Vitexin (apigenin-8-*C*-glucoside)	Antidiabetic (*α*-glucosidase inhibitors)	[17]

Table 1. Selected secondary metabolites from plants under the Malaysia's agriculture NKEA-EPP1.

Gajah (*Clinacanthus nutans* (Burm.f.) Lindau), and Pegaga (*Centella asiatica* (L.) Urb) [10]. It is noteworthy that a dietary supplement combining *Labisia pumila* and *Eurycoma longifolia* has reached the clinical trials investigating the effects on menopausal women and their quality of life (trial registration number NCT02269891) [11].

Not discounting many other important local plants, **Table 1** listed some of the important secondary metabolites from the agriculture NKEA-EPP1-select plants to illustrate the myriad of secondary metabolites responsible for various biological effects. The secondary metabolites range from flavonoids, quassinoids, phytosterols, and terpenoids.

3. Secondary metabolites from plant tissue culture

Overharvesting of medicinal plants for their secondary metabolites may lead to their disappearance from the natural habitats. Due to this, researchers turn to alternative and innovative methods to meet the increased demand for these natural products. In particular, plant tissue culture has become a well-established and attractive alternative for mass production of secondary metabolites through callus, suspension, and organ culture [18, 19]. Tissue culture itself is defined as the technique of maintaining plant tissue in vitro in a synthetic medium under controlled conditions, and it is reported to be extremely useful for commercial production of therapeutically important compounds [20].

There are many advantages of using plant tissue culture for producing metabolites including the (i) ability to improve the production of certain compounds within the plant cell using elicitors and plant hormones to manipulate the cultured cells [21], (ii) the ability to continuously produce secondary metabolites through propagation in sterile bioreactors independently of growth conditions such as soil content and microclimate [22], (iii) the ability of in vitro plant tissue culture to achieve higher rates of metabolism than the in vivo differentiated intact cells [22], and (iv) the ability to bypass the structural complexity of the plant organism rendering it to be a convenient tool in research studies [23].

Plant species	Type of plant culture	Secondary metabolites	Biological activities	Reference
***Boesenbergia rotunda* (Kencur)**	Embryogenic and non-embryogenic callus	Flavonoid (panduratin, pinocembrin, pinostrobin, cardamonin, and alpinetin)	Antimicrobial, antiulcer, antiviral, and antitumor activities	[24]
***Centella asiatica (L.) Urb* (Pegaga)**	Cell suspension	Triterpenoids (asiatic acid, madecassic acid, asiaticoside, and madecassoside)	Antibacterial, antimalarial, antiproliferative, and wound-healing properties	[25]
	Cell suspension	Flavonoid (quercetin, kaempferol, luteolin, and rutin)	Antibacterial, antiviral, antiallergic, antiplatelet, anti-inflammatory, antitumor, and antioxidant activities	[26]

Plant species	Type of plant culture	Secondary metabolites	Biological activities	Reference
***Eurycoma longifolia* Jack (Tongkat Ali)**	Hairy roots	Alkaloid (9-methoxycanthin-6-one)	Cytotoxicity activity against human breast cancer (MCF-7) and human lung cancer (A-549) cell lines	[27]
	Cell suspension culture	Alkaloid (9-hydroxycanthin-6-one and 9-methoxycanthin-6-one)		[28]
***Ficus deltoidea* Jack (Mas Cotek)**	Cell suspension	Flavonoids	Cardiovascular diseases and postpartum treatments, antidiabetic	[29]
***Justicia gendarussa* (Gandarusa)**	Callus, cell suspension	Phenolics	Antioxidant	[30]
***Morinda elliptica* (Mengkudu)**	Cell suspension	Phenolics (anthraquinones)	Antiviral, antimicrobial, cytotoxic, and antitumor-promoting and antioxidant activities	[31]
***Orthosiphon stamineus* (Misai kucing)**	Cell suspension	Phenolics	Antioxidant	[32]
***Pogostemon cablin* (Nilam)**	Cell suspension	Terpene (patchouli alcohol)	Nausea, diarrhea, and headache	[33]

Research conducted in various institutions in Malaysia.

Table 2. Different types of plant tissue culture and their secondary metabolites.

The current evaluation of secondary metabolite compounds in various culture types of medicinal plants available in Malaysia is summarized in **Table 2**. Most of these studies involved with the cell suspension culture compared to other types of plant culture that are also frequently used for secondary metabolite production such as hairy roots and shoot culture.

4. Case study: secondary metabolites in the prevention and treatment of dengue

4.1. Dengue fever

The dengue prevalence in Malaysia remains overwhelming, with increasing rate of incidence reported annually. According to the latest WHO report, in 2016, more than 375,000 suspected cases of dengue were recorded in the Western Pacific Region, 100,028 of which occurred in Malaysia [34]. The steady surge of cases over the years has prompted serious efforts from the government and the community, including intensive efforts in both vector control and elucidation of potential therapeutic agents. Although evaluation of several vaccines is currently in progress, the ambivalence of such treatment with regard to serotype

interference, incomplete protection, and dose sufficiency [35] makes the search for new antiviral agents imperative.

In Malaysia, the National Dengue Strategic Planning 2015–2020 (the Sixth Strategy: Dengue Research) highlights the importance of research aimed at enhancing the effectiveness, cost-effectiveness, sustainability, and the scale of existing interventions, as well as producing ideas and new methods, while promoting collaboration with relevant agencies. In this regard, it is anticipated that comprehensive research focusing on the local natural heritage, including the

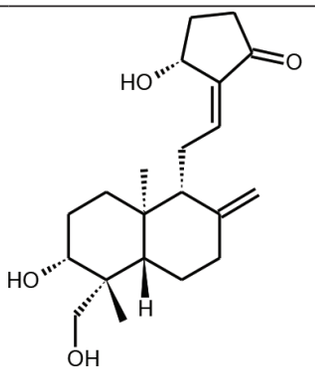
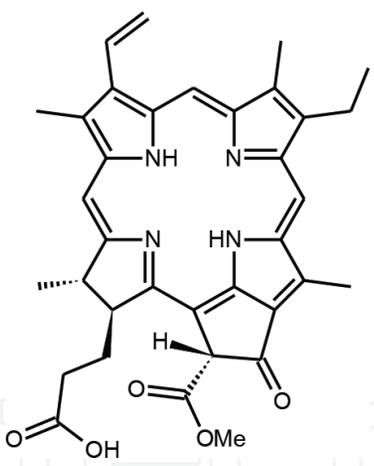
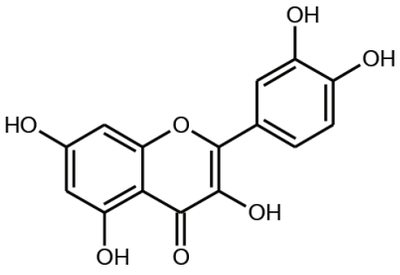
Plant	Secondary metabolites	Biological activity	Reference
Andrographis paniculata **(Hempedu Bumi)** leaves	 Andrographolide (class: terpenoid)	Anti-dengue activity against the primary dengue vector *Aedes aegypti*	[38]
Clinacanthus nutans (Burm.F) **(Belalai Gajah)** leaves	 Pheophorbide (class: chlorophyll)	Anti-DENV-2 activity by inhibiting the production of viral RNA and viral protein	[39]
Psidium guajava **(Jambu Batu)** leaves	 Quercetin (class: flavonoid)	Anti-dengue activity against different stages of DENV-2 infection and replication cycle	[40]

Table 3. Selected secondary metabolites from plants with effects against dengue or its vector.

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abundance of medicinal plants, should be prioritized, as this is deemed the best strategy for the development of new dengue fever treatment regimens.

4.2. Secondary metabolites from Malaysian plant species used for the prevention and treatment of dengue

Malaysia, due to its prolific nature and wide diversity of plant sources, offers a wide range of pharmaceutical options, with high potential as anti-DENV agents. Most of these plant species have not been studied extensively, and the existing research tends to focus on folklore medications. It is important to emphasize that the characterization of secondary metabolites responsible for anti-DENV activities is still very limited. Consequently, none of these medicinal plants have reached the clinical stage in the drug design pipeline, thus necessitating further verification studies. To date, only several secondary metabolites from a number of local Malaysian plant species have been identified and their chemical structures elucidated (**Table 3**), while many more remain to be explored. For instance, the effects of local plant extracts on in vitro dengue replication were recently documented [36], whereby *Hydrocotyle sibthorpioides* Lam. extracts showed variable effects on dengue viral replication, depending on the treatment, cell lines, and solvent types. In an earlier study, extract from *Phyllanthus* spp. also exhibits antiviral activity against DENV-2, which was supported by differential regulation of various hosts and viral proteins [37].

A summary of plant secondary metabolites found useful in dengue treatment and prevention is depicted in **Table 3**.

5. Challenges and future prospects

Despite a more concerted efforts and strategic approach to add value to the country's herbal industry, the desired output has not reached its optimum. The main challenges including the lack of good research documentation, monographs, standardization in farming practices (good agricultural practices, GAP), good laboratory practice (GLP), and development and commercialization of products still persist. Further, the increased harvesting of medicinal herbs raises the concerns about the extinction of plant populations and degradation of natural plant habitat causing shortage in plant raw materials which may affect the effort to reach high mass production of secondary metabolites. To this end, biotechnological applications such as plant tissue cultures have been recognized an alternative tool for scaling-up the production of secondary metabolites. However, challenges inherent in plant tissue cultures must be overcome in order for it to contribute to significant cost-effective production of secondary metabolites. This includes the understanding of the secondary metabolites and their metabolic pathways, identification of the highest yielding populations, control of specific gene expression and regulatory enzymes, and the use of economic sterile bioreactors. Leveraging the country's wealth of flora species, a continuous effort from the government, academia, and industry to further nurture and uphold the herbal industry is indispensable.

6. Conclusion

Malaysian tropical rainforests comprise a wide range of medicinal plants that have been screened for their secondary metabolites and potential biological and therapeutic effects. Selected plant species from the agriculture NKEA-EPP1 demonstrated numerous pharmacological activities which were attributed to the presence of biologically active secondary metabolites, namely, flavonoids, quassinoids, phytosterols, and terpenoids. The use of local plants toward combating the country's high-prevalent disease such as dengue could be further explored. While a lot of effort has been put in, further research and development into plant secondary metabolites are needed including using the plant tissue culture approach toward reaching high-value herbal industry.

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