Phytochemical screening of Malaysian Dipterocarpaceae species from Kuala Keniam, Taman Negara Pahang

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Abstract. The diverse family of Dipterocarpaceae is well-known for its timber as well as the phytochemical constituents which possess medicinal properties. In this preliminary study, 10 Dipterocarpaceae species namely Anisoptera laevis, Dipterocarpus crinitus, Dipterocarpus gracilis, Hopea nervosa, Hopea pubescens, Shorea hopeifolia, Shorea ovalis, Shorea parviflora, Vatica bella and Vatica maingayi were collected from Kuala Keniam, Taman Negara Pahang, Malaysia. The selected parts of the species were macerated in methanol and phytochemically screened to determine the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids. The results revealed that all crude methanolic extracts were found to contain tannins and terpenoids. Additionally, the crude extracts from the stem bark of D. gracilis and the leaves of H. pubescens were observed to contain alkaloids, flavonoids, reducing sugar, saponins, tannins as well as terpenoids. Further studies on all species are recommended in search for the bioactive chemical constituents that might be beneficial in the development of drug discovery.

1. Introduction
The National Park or locally known as Taman Negara is located in the centre of Peninsular Malaysia which stand in three states namely Pahang, Kelantan and Terengganu. The National Park is rich in flora and fauna which host more than 3000 species of plants and 150 species of mammals [1]. One of the common families found in the forest is Dipterocarpaceae, a family of hardwood [2] which is economically important in timber production. This aspect supports the Goal 15 of SDG-15, which is to protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests. Sustainable Development Goals (SDGs) is an urgent global call of action for all countries and research inclined to this goal will contribute more towards its achievement [3].
Dipterocarpaceae consists of 16 genera which includes *Anisoptera*, *Balanocarpus*, *Cotylelobium*, *Dipterocarpus*, *Hopea*, *Isoptera*, *Neobalanocarpus*, *Parashorea*, *Shorea*, *Upuna*, *Vateria* and *Vatica*. *Shorea* is the largest genus which consist of 194 species, followed by *Hopea* (104 species), *Dipterocarpus* (75 species) and *Vatica* (66 species). Meanwhile, *Anisoptera* is a small genus in the family with only 10 species were recorded. Table 1 shows vernacular names and the occurrence of selected Dipterocarpaceae species in Malaysia.

**Table 1.** Vernacular names and habitat of selected Dipterocarpaceae species in Malaysia

| Genus         | Vernacular names and habitat in Malaysia                                                                 |
|---------------|----------------------------------------------------------------------------------------------------------------|
| *Anisoptera*  | *A. laevis* is locally known as *mersawa durian* and *pengiran durian*. It occurs on lowland to hill forests up to 900 m altitude. |
| *Dipterocarpus* | *D. crinitus* is locally known as *keruing mempelas*. The habitat is at lowland and hill forests up to 900 m altitude.  
|               | *D. gracilis* is locally known as *keruing kesat*. It can be found in valleys, hill slopes and ridges from lowland to hill forests up to 800 m altitude and in seasonally dry tropical rain forests. |
| *Hopea*       | *H. pubescens* is locally known as *merawan bunga*. The habitat is at lowland dipterocarp forests, on well drained flat land and low hills.  
|               | *H. nervosa* is locally known as *merawan jangkang*, *selangan jangkang* and *luis jangkang*. It can be found at lowland to hill forests up to 600 m altitude. |
| *Shorea*      | *S. hopeifolia* is locally known as *damar siput jantan*, *seraya kuning jantan* and *lun siput jantan*. It occurs at lowland to upper hill forests at altitudes up to 1200 m, showing a preference for the vicinity of streams.  
|               | *S. ovalis* is locally known as *meranti kepong* and *seraya kepong*. The habitat is at lowland and hill forests.  
|               | *S. parvifolia* is locally known as *meranti sarang punai* and *seraya punai*. It can be found at lowland to hill forests. |
| *Vatica*      | *V. bella* is locally known as *resak keluang*. It occurs on undulating land below 250 m altitude.  
|               | *V. maingayi* is locally known as *resak lidi* and *resak daun merah*. The habitat is at lowland forests up to 200 m altitude. |

*Source: FRIM [4]*

In the past decades, the old folks used various parts of the Dipterocarps to treat illnesses. The wood of *Dipterocarpus littoralis* was used as a building material and furniture industry. Meanwhile, the stem bark of this species was used as a traditional remedy to treat diseases like diarrhoea, diabetes and malaria [5]. The leaves and bark of *Shorea robusta* were used to treat wounds, ulcers, cough and headache while the fruits were important in treating dermopathy and tubercular ulcers [6].

Rose and co-workers [7], reported on the antioxidant and antibacterial activities of methanol and aqueous extracts of *Hopea ponga*. Additionally, the leaf, bark and twig extracts of *Dipterocarpus alatus*
demonstrated significant cytotoxicity and antioxidant activity against the U937 cell lines [8]. The hexane, chloroform and ethyl acetate extracts from the flowers of D. intricatus exhibited antioxidant activity on DPPH radical, antibacterial ability against B. cereus, E. coli, P. aeruginosa, S. enteritidis, S. typhimurium and S. aureus as well as cytotoxicity on Hep G2 cell lines [9].

In this study, we are reporting the phytochemical screening of 10 Dipterocarp species that have been collected from Kuala Keniam, Taman Negara Pahang, Malaysia namely Anisoptera laevis, Dipterocarpus crinitus, D. gracilis, Hopea nervosa, H. pubescens, Shorea hopeifolia, S. ovalis, S. parviflora, Vatica bella and V. maingayi.

2. Experimental

2.1 Plant Materials
Fresh samples of A. laevis (leaves, stem bark and twigs), D. crinitus (leaves and stem bark), D. gracilis (stem bark), H. nervosa (stem bark), H. pubescens (leaves and twigs), S. hopeifolia (leaves and stem bark), S. ovalis (leaves and stem bark), S. parviflora (stem bark), V. bella (leaves and twigs) and V. maingayi (leaves and twigs) were collected from Kuala Keniam in Taman Negara, Pahang. The plant samples were authenticated by the botanist from Forest Biodiversity Division, FRIM and the voucher specimens of A. laevis (FRI 94375), D. crinitus (FRI 92041), D. gracilis (FRI 94000), H. pubescens (FRI 94236), S. ovalis (FRI 94354), S. parviflora (FRI 93938) and V. bella (FRI 92045) were deposited at FRIM while V. maingayi (KK5-25) was deposited at UiTM Jengka.

2.2 Preparation of extracts
The leaves, stem bark and twigs of the species were air-dried for two weeks and ground in granulated form. The granules of each sample were macerated in methanol at room temperature for 24 hours and repeated three times. The extracts were filtered and evaporated to dryness using a rotary evaporator to yield crude methanol extracts. The crude extracts were stored in capped vials before being screened.

2.3 Phytochemical screening
The crude extracts were dissolved in ethanol and 3 mL of the crude extracts was transferred into the boiling tubes respectively. The crude extracts were phytochemically screened to detect the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and reducing sugar using standard methods with slight modifications.

Alkaloids (Mayer’s Test)
Approximately 1 mL of Mayer’s reagent was added into a boiling tube containing crude extract. The formation of cream colour precipitate indicated the presence of alkaloids [10].

Flavonoids (Shinoda Test)
A magnesium strip was added into a boiling tube containing crude extract followed by few drops of hydrochloric acid, HCl. The appearance of reddish-pink or yellow precipitate indicated the presence of flavonoids [10].

Reducing sugar (Fehling’s Test)
A 2.5 mL of each Fehling’s solutions (A and B) were mixed before being added into a boiling tube containing crude extract. The sample were then boiled in a water bath for 5 minutes. The appearance of brick-red precipitate at the bottom of the boiling tube indicated the presence of reducing sugar [11].
Saponins
Approximately 5 mL of distilled water was added into a boiling tube containing crude extract and closed with parafilm. The boiling tube was shaken vigorously for a stable persistence froth. The formation of a stable foam indicated the presence of saponins [11].

Tannins (Ferric Chloride Test)
A boiling tube containing crude extract was added with 5 mL of ferric chloride, FeCl₃ solution. The presence of brownish-green or a blue-black colour indicated the presence of tannins [10].

Terpenoids (Salkowski Test)
A 2 mL of chloroform was added into a boiling tube containing crude extract followed by the addition of 3 mL of concentrated sulphuric acid, H₂SO₄ to form a layer. The formation of a reddish-brown colour of the interface indicated the presence of terpenoids [12].

3. Results and Discussions
Dipterocarpaceae family is well-known for its quality of wood which is valuable in timber production. Besides, it has shown important role in folklore medicine for treating ear problems, diarrhoea, hemorrhoids, wound and anthelmintic [13-15]. The healing properties might be due to the presence of secondary metabolites in the plant. Thus, the qualitative preliminary screening on the crude methanol extracts of A. laevis (leaves, stem bark and twigs), D. crinitus (leaves and stem bark), D. gracilis (stem bark), H. nervosa (stem bark), H. pubescens (leaves and twigs), S. hopeifolia (leaves and stem bark), S. ovalis (leaves and stem bark), S. parviflora (stem bark), V. bella (leaves and twigs) and V. maingayi (leaves and twigs) was executed to detect the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids.

Based on the observations in Table 2, the presence of the classes of compounds are categorised as significantly present (+++), moderately present (++), present (+) and not present (−). The crude extracts of D. gracilis (stem bark) and H. pubescens (leaves) demonstrated the presence of all classes of compounds. Meanwhile, the results from the Salkowski test shows that terpenoids present in all crude extracts. Most crude extracts demonstrated high (+++) amount while three crude extracts namely D. crinitus (stem bark) and S. hopeifolia (leaves and stem bark) showed moderate amount (++) of terpenoids. The phytochemical screening reported on the crude methanol extracts from the resin of S. robusta [16] and the leaves of V. diospyroides [17], H. ponga [18] and D. Zeylanicus [19] demonstrated the presence of terpenoids which is good agreement with our findings. Terpenoids is one of the important classes of compounds that possess various biological activities. The anticancer drug, Taxol as well as the antimalarial drug, artemisinin are the examples of terpenes with established medical application [20]. Additionally, monoterpenes which are popular as main constituents in essential oils, floral and scents possess antioxidant, anticonvulsant, antimicrobial, antitussive, anti-inflammatory and antitumor activities [20, 21]. It is reported that the hydrophobicity of monoterpenes is mainly related to antimicrobial and antitussive activities [22].

All crude extracts screened were found to contain tannins ranging from low (+) to significant (+++) amount. These results are consistent with the previous studies which showed that tannins are found in most part of plants such as in the resin of S. robusta [16] and the leaves of V. diospyroides [17], H. ponga [18] and A. scaphula [23]. Additionally, the leaves, seeds and heartwood of D. Zeylanicus also displayed high amounts of tannins while the resin contained tannins in moderate amounts [19]. Tannins exhibited antifungal activity against Aspergillus niger and antioxidant properties by showing the ability to scavenge the free radicals and high ferric reducing power [24].
Additionally, most crude extracts contain low (+) to significant (+++) amount of reducing sugar while two crude extracts gave negative results in Fehling’s test which are the leaves of *S. ovalis* and *V. maingayi*. The presence of reducing sugar in Dipterocarpaceae species was shown by previous studies on the leaves, seeds, heartwood and resin of *D. Zeylanicus* [19]; leaves, bark and twigs of *D. alatus* [8] as well as the leaves of *H. ponga* [18] and *A. scaphula* [23].

There are twelve crude extracts exhibited low (+) to high (+++) amount of saponins except for the crude extracts of *D. crinitus* (leaves), *H. pubescens* (twigs), *S. hopeifolia* (stem bark), *S. ovalis* (stem bark), *V. bella* (twigs) and *V. maingayi* (twigs). Previous findings on the crude extracts from the leaves of *V. diospyroides* [17], *H. ponga* [18] and *A. scaphula* [23] displayed the presence of saponin while the branch of *V. diospyroides* [17] showed no result. Saponin has bitter and unpleasant taste with colourless amorphous form. This class of compound has different solubility due to certain conditions such as temperature, composition and pH [25]. Saponins possess significant biological activities such as adjuvant activity, cholesterol binding activity, anti-inflammation, antitumor and cardiovascular protection [25-26].

On the other hand, only *A. laevis* (stem bark and twigs), *D. crinitus* (leaves), *D. gracilis* (stem bark), *H. pubescens* (leaves), *S. hopeifolia* (leaves and stem bark), *S. ovalis* (leaves) as well as *V. maingayi* (leaves and twigs) exhibited the presence of alkaloids in Mayer’s test but in low (+) amount. In *D. alatus*, among four (leaves, bark, twigs and oleo resin) plant parts tested, only the bark showed the presence of alkaloid [8]. Moreover, it was also reported that the crude methanolic extract from the leaves of *D. Zeylanicus* did not contain alkaloid while the seeds, heartwood and resin showed low amount of alkaloid [19]. Alkaloids are organic compounds containing nitrogen which can be found in the root, stem, rhizome, fruit and bark of the plants [27]. They demonstrated anti-inflammatory, anticancer, analgesics, antimicrobial and antifungal activities [28].

There are only eight crude extracts which are *D. crinitus* (leaves and stem bark), *D. gracilis* (stem bark), *H. nervosa* (stem bark), *H. pubescens* (leaves), *S. ovalis* (leaves and stem bark) and *S. parviflora* (stem bark) displayed low (+) amount of flavonoids while the remaining gave negative results in Shinoda test. It was found that the leaves part contains flavonoids which is supported by the report on the leaves of *V. diospyroides* [17], *H. ponga* [18] and *A. scaphula* [23]. Flavonoids which are commonly found in food such as onion, broccoli, tea, cherry and lemon were reported to exhibit antioxidant, anticancer, prevention of cardiovascular disease and antiviral activities [29-30].
Table 2. Results of phytochemical analyses of the selected 10 Dipterocarpaceae species

| Species (Part) | Alkaloids | Flavonoids | Reducing sugar | Saponins | Tannins | Terpenoids |
|---------------|-----------|------------|----------------|----------|---------|------------|
| *A. laevis* (leaves) | - | - | + | ++ | + | +++ |
| *A. laevis* (stem bark) | + | - | ++ | + | + | +++ |
| *A. laevis* (twigs) | + | - | +++ | + | ++ | +++ |
| *D. crinitus* (leaves) | + | + | + | - | ++ | +++ |
| *D. crinitus* (stem bark) | - | + | + | + | ++ | ++ |
| *D. gracilis* (stem bark) | + | + | + | + | +++ | +++ |
| *H. nervosa* (stem bark) | - | + | + | +++ | ++ | +++ |
| *H. pubescens* (leaves) | + | + | ++ | ++ | +++ | +++ |
| *H. pubescens* (twigs) | - | - | +++ | - | + | +++ |
| *S. hopeifolia* (leaves) | + | - | + | + | ++ | ++ |
| *S. hopeifolia* (stem bark) | + | - | +++ | - | +++ | ++ |
| *S. ovalis* (leaves) | + | + | - | + | + | +++ |
| *S. ovalis* (stem bark) | - | + | + | - | +++ | +++ |
| *S. parviflora* (stem bark) | - | + | + | +++ | ++ | +++ |
| *V. bella* (leaves) | - | - | + | + | ++ | +++ |
| *V. bella* (twigs) | - | - | + | - | + | +++ |
| *V. maingayi* (leaves) | + | - | - | ++ | + | +++ |
| *V. maingayi* (twigs) | + | - | + | - | + | +++ |

- not present, + present, ++ moderately present, +++ significantly present
4. Conclusions
The phytochemical screening of selected Dipterocarpaceae species revealed the presence of all classes of compounds tested in the crude extracts of *D. gracilis* (stem bark) and *H. pubescens* (leaves). Additionally, all crude extracts tested were found to contain large amounts of terpenoids and tannins and low to high amounts of alkaloids, flavonoids, reducing sugar and saponins. The results indicated that the species from Dipterocarpaceae have the potential source of bioactive compounds. To the best of our knowledge, this is the first study on the phytochemical properties of Malaysian Dipterocarpaceae namely *A. laevis, D. crinitus, D. gracilis, H. nervosa, H. pubescens, S. hopeofila, S. ovalis, S. parviflora, V. bella* and *V. maiungayi*.

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