Screening of Phytochemicals from the Ethanolic Extracts of *Gnetum gnemon*, *Gnetum latifolium* and *Cynometra malaccensis* of Kuala Keniam, Pahang

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**Abstract.** The diversity of plants has led to various findings in the medicinal world. The abundance of bioactive compounds existing in the plant is the main factor contributing to its application in pharmaceutical industries. *Gnetum gnemon*, *Gnetum latifolium* and *Cynometra malaccensis* were collected from the forest area, Stesen Penyelidikan UiTM -Perhilitan, Kuala Keniam, Taman Negara, Pahang. This project focussed on profiling the bioactive constituent through phytochemical screening via Shinoda, Mayer, saponin, tannin, and terpenoid tests from the ethanolic extract of stem bark, stem, leaves and twigs of the three species. The results displayed that all plant parts showed the presence of flavonoids, terpenes and tannins. These compounds might contribute to the good properties of the plants.

1.0 Introduction

Malaysia has been classified as a megadiverse country due to its location in the tropical belt. Peninsular Malaysia includes about 10,000 higher and approximately 2000 lower plants, with approximately 16% claiming therapeutic properties [1]. Our lush tropical rain forests contain an abundance of chemically diverse compounds with therapeutic properties. Taman Negara, a protected tropical forest, is unexplored primarily and represents an enormous opportunity to discover beneficial plants.

The Gnetaceae families are comprised of a single genus, *Gnetum*. Numerous species in the genus have been used in traditional medicine to treat rheumatoid arthritis, bronchitis, and asthma and are a source of oligostilbenoids. Previous research has indicated that the chemicals extracted from *Gnetum* species have high bioactivity due to the presence of oligostilbene compounds, which are useful as chemotaxonomic agents, metabolites, and constitutive defence agents. They have been found to demonstrate a broad range of biological activities due to their possible pharmacological qualities, including anti-cancer, anti-inflammatory, and antioxidant effects [2].
Fifteen species of *Gnetum* are present in Malaysia's rainforests below 1800 metres. Belinjau or melinjau (*G. gnemon var. gnemon*) is the most well-known, as it is the sole tree species in the genus in the country. Numerous species in the family have been used in traditional medicine to treat conditions such as arthritis, bronchitis, and asthma. The leaves and fruits are also consumed [3]. Nutritionally, certain species within the family are significantly protein- and mineral-dense [4]. Stilbenoids are known to be present in *Gnetaceae* plants [5], and they have been shown to exert a wide variety of biological functions.

The Fabaceae family, which includes beans and peas, and the Anacardiaceae family, which includes cashews, are two plant families that are commonly reported to possess flavonoids. Several components of plants belonging to the Fabaceae family are used in traditional medicine to treat various diseases. Simultaneously, edible nuts from the Anacardiaceae family have a reputation for being beneficial to the brain. *Cynometra*, one of the Fabaceae genera, is claimed to contain alkaloids but has not been thoroughly explored for their therapeutic effects due to the absence of many species from folk medicine [6, 7].

The abundance of the bioactive metabolite that existed in the plant has made it possible in conducting preliminary studies to examine the bioactive constituent using phytochemical screening such as Shinoda, Mayer, saponin, tannin, and terpenoid tests from the ethanolic extract of stem bark, stem, leaves and twigs of the three species.

2.0 Experimental

2.1 Plant Materials
Three plant specimens were collected from the Kuala Keniam Forest in Taman Negara, Pahang. The botanist from the Forest Research Institute Malaysia (FRIM) identified the plants, and voucher specimens were placed with the voucher number in the FRIM Herbarium.

2.2 Procedure for Extraction
The dirt was removed from all plant material by gently cleaning with tap water and dried at room temperature for two weeks. The dried leaves were ground into small pieces and processed into a powder using a grinder. Ethanol was utilised as the solvent for extraction. For 72 hours at room temperature, the sample was macerated in ethanol at a ratio of 100g sample to 1-litre ethanol. Filtration of the ethanolic extract was performed using Whatman No. 1 filter paper (32.0 cm). The extracts were concentrated by evaporating them under reduced pressure at 20 mbar/50° C using a rotary evaporator. The extracts were kept at a temperature of 5° C until utilised.

2.3 Screening for Phytochemical Compounds
A small amount of dried crude methanol extracts of *G. gnemon*, *G. latifolium*, and *C. mallacensis* were dissolved in ethanol and subjected to screening assays such as the Shinoda, Mayer, saponin, tannin, and terpenoid tests. This screening test was used to determine the sample's flavonoid, alkaloid, saponin, tannin, and terpene content.

2.3.1 Shinoda Test
To determine the presence of flavonoid compounds, the Shinoda test was used. A sample volume of 2 ml was transferred to a test tube. A few cut magnesium ribbons and a few drops of HCl solution were added to the test tube. A pink or red solution indicated the presence of a flavonoid component in the sample.

2.3.2 Mayer's Test
Mayer's reagent was added to the aqueous extract to test the presence of alkaloids. With a few drops of Mayer's reagent, the sample volume of 3 ml was put into a test tube. A cream colour developed in the samples suggested the presence of an alkaloid component.

2.3.3 Saponin Test
A 0.5 ml aqueous solution of the sample was diluted with 5 ml distilled water in a test tube, and the test tube's mouth was covered with paraffin film. The test tube was shaken briskly until the formation of foam was observed. If the foaming persists for 15 minutes, saponin is present in the sample.
2.3.4 Tannin Test
0.5 ml of sample was combined with 3.5 ml of distilled water in a test tube for the tannin test. Afterwards, 0.5 ml of the diluted sample was transferred to a test tube, adding 0.5 ml of FeCl₃. The presence of tannin in the sample was shown by forming a green or blue colour.

2.3.5 Terpenoid Test
To ascertain the existence of terpenoids, a terpenoid test was performed. A sample volume of 0.5 ml was transferred to a test tube. The test tube was filled with 2 ml of CHCl₃ and 3 ml of concentrated H₂SO₄. The formation of a reddish-brown colour suggested the existence of terpenoid molecules.

2.3.6 Reducing Sugar Test
Approximately 5.0 ml of extract was added to boiling Fehling A and B solutions in a test tube. The presence of reducing sugar was established by the appearance of a brick-red precipitate at the bottom of the test tube.

3.0 Results and Discussion
The possibility of discovering novel chemical constituents and the possible pharmacological applications of constituents that could serve as lead compounds in developing new drug entities justifies the phytochemical studies on these three Malaysian plant species. These plants were collected and screened to determine the type of constituents present in these plants (Table 1).

| Species                  | Part       | Shinoda Test | Mayer Test | Reducing Sugar Test | Terpene Test | Tannin Test | Saponin Test |
|--------------------------|------------|--------------|------------|--------------------|--------------|-------------|--------------|
| Gnetum gnemon            | Leaves     | +++          | -          | -                  | ++           | ++          | -            |
|                          | Twigs      | ++           | -          | -                  | +            | +           | -            |
| Gnetum latifolium        | Leaves     | ++           | -          | -                  | +            | +           | -            |
|                          | Seed       | +            | -          | -                  | +            | +           | -            |
|                          | Twig       | +            | +          | -                  | +            | +           | -            |
|                          | Stem       | +            | +          | -                  | +            | +           | -            |
| Cynometra mallacensis    | Leaves     | ++           | -          | -                  | +++          | +++         | -            |
|                          | Twigs      | +            | -          | -                  | +++          | +           | -            |
|                          | Stembark   | ++           | -          | -                  | ++           | +           | -            |

G. gnemon (leaves and twigs), G. latifolium (leaves, seed, twigs, and stem), and C. mallacensis (leaves, twigs, stem bark) crude ethanol extracts were screened for the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids.

All three plants showed the presence of flavonoids with the highest concentration displayed in the leaves of G. gnemon (3+), followed by the twigs of G. gnemon, the leaves of G. latifolium, the leaves and stembark of C. mallacensis, each displaying 2+ for the presence of flavonoids. The rest of the plant parts showed 1+ for flavonoids. Gnetum is well-known for its high polyphenolic content. According to the phytochemical investigation, Gnetum species have a high concentration.
of oligostilbenes, flavonoids, and alkaloids. Apart from the components, phenylpropanoid and its derivatives, lignans, terpenoids, and a few phenolic acids are found in *Gnetum* [8]. Wallace and Morris (1978) [9] were the first to report the presence of C-glycosylflavones, swersitin, isoswersitin, isovitexin, vicenin 2, swertiajaponin, and isowertiajaponin, as well as O-glucosides, 7-O-glucosylisovitexin, in the leaves of *G. gnemon*. Recent studies on *Cynometra* successfully isolated several types of flavonoids compounds such as xanthotoxin, Fraxetin, capensine, naringenin, malvidin, cyanidin, amorphigenin, nobiletin, isorhamnetin, epigallocatechin, gallate, apigenin and oenin [10].

The leaves and twigs of *C. mallacensis* showed high content of terpenes (3+), followed by the stem bark of *C. mallacensis* and the leaves of *G. gnemon* (2+), while the rest of the extracts contain only 1+ for the presence of terpenes. In a study of the volatile oils from the roots of *C. megalophylla*, monoterpenes, sesquiterpenes, and aliphatic oil components were discovered [11]. A triterpene, β-daucosterol, was reported from both lianas of *G. pendulum* [12] and *G. montanum* [13].

High content of tannin (3+) can be observed in the leaves of *C. mallacensis*. In comparison, the stem bark of *C. mallacensis* and leaves of *G. gnemon* showed a moderate amount of tannins. In various parts of *C. iripa*, polyphenols, tannins, flavonoids, and saponins are detected [14]. Abd Aziz and Iqbal (2013) [15] had screened the presence of tannins, terpenoids, saponins, and flavonoids in all *C. cauliflora*. Additionally, Ado et al. (2014) [16] examined the active constituents of *C. cauliflora* leaf extract. They isolated 18 compounds, including a procyandin trimer, tetramer, hexamer, and a taxifolin pentoside, catechin, vitexin, isovitexin kaempferol hexoside, quercetin, pentoside, quercetin hexoside, apigenin-6-C-glucoside, and kaempferol-coumaroyl.

Numerous studies on the presumptive identification of chemical elements in *Cynometra* plants have been described in various works. The abundance of tannin and terpene in the screening test of *C. mallacensis* was bolstered by data from numerous studies on the presumptive identification of chemical elements in *Cynometra* plants. The existence of alkaloids, flavonoids, terpenoids, and other chemicals in *Cynometra* plants, as described in peer-reviewed journals, should arouse interest in the species' pharmacological investigation.

The rest of the tests showed no presence of saponin and reducing sugar in all plant parts. No alkaloids were detected except minor content in the twig and stem of *G. latifolium*. According to data from the CHEMnetBASE Dictionary of Natural Products database, around 163 bioactive compounds have been identified and isolated from *Gnetum*, while only 11 bioactive compounds have been identified and isolated from *Cynometra*, where all of it is an alkaloid compound from two cynometra species, *Cynometra lujae* and *Cynometra anantha* [17].

### 4.0 Conclusions

The phytochemical screening on *Gnetum gnemon*, *Gnetum latifolium* and *Cynometra mallacensis* showed the presence of flavonoids, terpenes and tannins in all plant parts with various concentrations. No saponin, reducing sugar and alkaloid were detected in all parts except for the minor alkaloid content in the twigs and stems of *G. latifolium*. The high content of flavonoid and terpene in the leaf and twig of *G. gnemon* and the leaf and twig of *C. mallacensis* warrant further study in the isolation of chemical constituents and the bioassays from these two plants.

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