UNIVERSITI TEKNOLOGI MARA

CHEMICAL CONSTITUENTS OF *Garcinia eugenifolia* AND *Garcinia nitida* AND THEIR CYTOTOXIC ACTIVITIES AND ANTIOXIDANT PROPERTIES

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5. Report

5.1 Proposed Executive Summary

There are several local Malaysian *Garcinia* species which have ethnobotanical uses but so far very few studies have been carried out to link these folkloric uses with the phytochemistry of these plant species. It would be interesting therefore, to develop phytochemical data of two such Sarawakian species which are *Garcinia eugenifolia* and *Garcinia nitida* which have not been reported before.

Some species of *Garcinia* have been shown to possess interesting biological activities such as cytotoxic, antibacterial, antioxidant and anti-cancer activities. Leaves and stem bark samples of *Garcinia eugenifolia* and *Garcinia nitida* will be extracted and purified using standard protocols which involve the conventional extraction technique such as chromatographic methods. Analyses for structural elucidations of pure bioactive compounds will involve the usual spectroscopic techniques such as NMR, FTIR, UV and MS.

The isolation and identifications of these natural products will lead the researchers to establish a profile of chemical and biological activities of the extract for standardisation and product development. The outcome of this project, which is a documentation of medicinal plants rich in active compounds, is for further investigation for their potential uses in drug development.

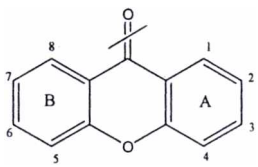
5.3 Introduction

Garcinia (Guttiferae) is distributed in Thailand, India, Sri Lanka, Myanmar, Indonesia, Malaysia, the Philippines and China. People in these countries often use *Garcinia* for traditional medicines including the treatment of abdominal pain, dysentery, diarrhea, suppuration, infected wound, leucorrhoea, and chronic ulcer and gonorrhea (Jayaprakasha *et al.*, 2006). Furthermore, *Garcinia* exhibited an anti-inflammatory (Gopalakrishnan *et al.*, 1997), antibacterial activity against *Staphylococcus aureus* (Sakagami *et al.*, 2005) and *Helicobacter pyroli* (Mahabusarakum *et al.*, 1983), and antitumour and antioxidant abilities (Williams *et al.*, 1995).

The *Garcinia* species are rich sources of mangostin, tannin, xanthone, isoflavone, flavone and other bioactive substances (Deachathai *et al.*, 2005; Jung *et al.*, 2006). Extensive researches have shown that some bioactive compounds from the *Garcinia* species exhibited a wide range of interesting biological and pharmacological activities such as cytotoxicity, anti-cancer, antibacterial and antioxidant activities. Such research findings are vital to the biotechnology industry in Malaysia as the country aims to be a global player in the natural product sector (Ismail, 2001). However, there are no studies on *Garcinia eugenifolia* and *Garcinia nitida* from Sarawak. Therefore, it is expected that there will be active compounds with potential cytotoxic and antioxidant activities of *Garcinia eugenifolia* and *Garcinia nitida* from Sarawak.

5.4 Brief Literature Review

Previous chemical studies have revealed that the *Garcinia* species are rich sources of xanthones (**1**), particularly oxygenated and prenylated xanthones; flavanoids (**2**) such as 3,8''- linked biflavanoids; benzophenones (**3**), especially prenalylated benzophenones as well as triterpenoids.



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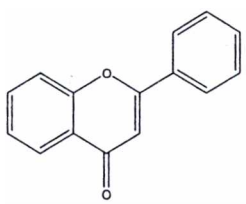

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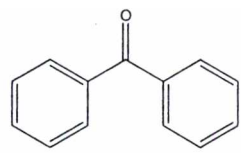
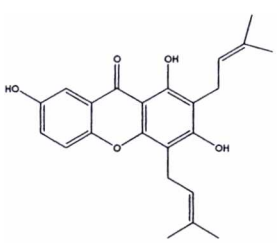


(3)

For instance, previous phytochemical studies on some *Garcinia* species including *G. cambogia, G. bracteata, G. indica, G. cowa, G. paucinervis and G. kola* have isolated xanthones, benzophenones, anthocyanins, mangostin and flavonoids (Milena *et al.*, 2010; Zhi *et al.*, 2010; Chetan *et al.*, 2010; Adaramoye *et al.*, 2005; Gao *et al.*, 2010), whereas chemical studies on Malaysian *G. penangiana*, *G. cantleyana*, *G. atroviridis* have revealed the presence of xanthones, xanthonoids and quinines (Jabit *et al.*, 2007; Khalid *et al.*, 2007; Dharma *et al.*, 2000).

Garcinia mangostana and *Garcinia subelliptica* are the two species that have been well studied. There is no previous recorded work on *Garcinia eugenifolia*, neither in its chemistry constituents nor the biological activities. Isolation on the stem bark extract of *Garcinia nitida* has led to the isolation of five xanthones: 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone **(4)**, osajaxanthone **(5)**, inophyllin B **(6)**, 3-isomangostin **(7)** and rubraxanthone **(8)** (Ee *et al.*, 2007).



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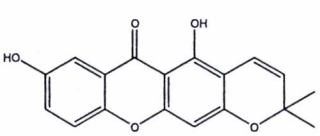

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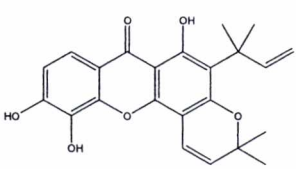

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5.5 Methodology

5.5.1 Plant Materials

The samples of *Garcinia nitida* and *Garcinia eugenifolia* were collected from a mixed dipterocarp forest, Semenggok. The herbarium voucher specimens of *Garcinia nitida* (UiTM 3001) and *Garcinia eugenifolia* (UiTM 3003) were identified and authenticated by a plant taxonomist at the Forestry Research Centre (FRC), Sarawak. The voucher specimens were kept at Universiti Teknologi MARA (UiTM) Sarawak.

All the plant parts were air-dried at room temperature, and the plants samples were cut into smaller pieces before grounded into fine powder.

5.5.2 Extraction and Isolation

Generally, all crude extracts of the studied plant samples were prepared using the cold extraction method. A few isolation techniques such as liquid vacuum chromatography, column chromatography, radial chromatography and preparative thin layer chromatography were used to isolate the compounds. These chromatography methods were carried out using various suitable solvents system which were based on the TLC profiles to afford the pure compounds. Silica gel 60 GF_{254} (MERCK 1.007730) and silica gel 60 PF_{254} (MERCK 1.007749) were used for liquid vacuum and radial chromatography respectively. The column chromatography was performed using silica gel Merck Kieselgel 60 Art. No. 9385.1000 of particle size 0.040 – 0.063 mm (230-400 mesh) while preparative thin-layer chromatography (PTLC) was performed on Whatman glass-backed plates coated with silica gel 60 with a fluorescent indicator. Sigma Lipophilic Sephadex LH-20 was used to further purify the compound when necessary. Spots and bands for compounds on TLC, PTLC and radial chromatography were detected using UV lights.

5.5.2.1 *Garcinia eugenifolia L.*

The dried and powdered twigs (1.30 kg) and roots (2.30 kg) of *Garcinia eugenifolia L.* were macerated in hexane, ethyl acetate and methanol, and allowed to stand for 24 hours at room temperature (28°C). The solvent was then removed by filtration, and fresh solvent was added to the plant material. The extraction process was repeated three times on a 2-day interval. The collected extracts were filtered and evaporated to dryness under reduced pressure, yielding 7.0 g of crude hexane extract, 28.6 g of crude ethyl acetate extract and 19.5 g of crude methanol extract from the twigs and 12.0 g of crude hexane extract, 45.7 g of crude ethyl acetate