Antiproliferative activity of five *garcinia* species collected in Sabah, Malaysian Borneo against estrogen receptor- human breast carcinoma (MCF-7) cell line

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Abstract. *Garcinia* species are well-known for their unique properties of having natural secondary metabolite compounds called xanthone as well as their ethnomedicinal values such as antioxidant, anticancer, anti-inflammatory and antibacterial properties. The study was conducted to investigate the antiproliferative activity of peel, flesh and seed extracts of *G. dulcis*, *G. parvifolia*, *G. nitida*, *G. mangostana* var. *mangostana* and *G. cambogia* collected from Malaysian Borneo (Sabah) against estrogen receptor-positive human breast carcinoma (MCF-7) cells. The antiproliferative activity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results showed that *G. dulcis* seed induced strongest antiproliferative activity against MCF-7 cancer cell line with the IC\(_{50}\) value of 2.5±0.0 µg/ml, followed by *G. dulcis* flesh, *G. mangostana* var. *mangostana* peel and *G. dulcis* peel with IC\(_{50}\) values of 9.33 ± 3.21, 11.17 ± 1.04 and 17.67 ± 2.08 µg/ml, respectively. Meanwhile, the IC\(_{50}\) value for *G. cambogia* peel was 56.67 ± 10.5 µg/ml. No IC\(_{50}\) value was detected in all parts of *G. parvifolia* and *G. nitida* at concentration tested (<100 µg/ml). Overall, this study clearly showed that the whole fruit of *G. dulcis* displayed potent cytotoxic effect by inducing antiproliferative activity at low concentration. Further studies are needed in the future to develop this fruit as pharmaceutical and nutraceutical product for the treatment and prevention against cancer.

1. Introduction
Cancer is currently one of the leading causes of death worldwide. In 2008, it is estimated about 12.7 million cancer cases have been reported of which 7.6 million deaths are linked to cancer. Breast cancer is the most frequently diagnosed cancer and number one leading cause of cancer death in women worldwide, accounting for 23 % (1.38 million) of the total new cancer cases and 14 % (458,400) of the total cancer deaths in 2008. The incidence of breast cancer varies greatly around the world in which half of the breast cancer cases compromising 60 % of deaths are estimated to occur in economically developing countries [1]. In 2006, breast cancer is the most common cancer among female and the most important cancer among population regardless of sex in Peninsular Malaysia of which the incidence is
highest among Chinese (46.4 per 100,000), followed by Indian (38.1 per 100,000) and Malay (30.4 per 100,000) [2].

There are few factors associated with high risk of breast cancer disease. The nulliparous woman (never given birth to a child) has 15.3 times higher chances of breast cancer compare to those who have more than two children, 2.1 times higher in woman with obesity, 4.3 times higher in woman with family history of breast cancer and 2.5 times higher in woman who previously used contraceptive pills [3]. Cancer survivals are less in developing countries, most likely because of late stage diagnosis and limited access to timely and standard treatment [1]. A cohort retrospective study by Ibrahim et al. [4] showed that the overall survival rate of breast cancer patients was 48.5 %, which was lower compared to two previous cohort studies done by Hisham and Yip [5] and Mohd Taib [6] with the values of 58.4 % and 75.7 %, respectively at the same local hospital (Hospital Kuala Lumpur). Based on ethnicity, Malays are found to be the poorest survivals due to the late stage diagnosis [4]. Norsa’adah et al. [7] also reported that poor knowledge of awareness on breast cancer, fear of consequences, denial of disease and attitude of ‘wait and see’ are among factors that enhance the late presentation among woman with breast cancer.

Recently, the exploitation of natural compounds from fruits for medicinal purposes has drawn much attention mainly due to the presence of secondary metabolites that potentially possessed antioxidant and anticancer properties. Tones of epidemiological research suggested that the consumption of natural antioxidant such as polyphenol-rich food, fresh fruits, vegetables or teas have protective effects against several diseases such as cancer, rheumatoid arthritis as well as in the degenerative process related to aging, including Parkinson’s and Alzheimer’s diseases [8].

*Garcinia* species of the Clusiaceae family collected in Sabah have been recognized as having pharmaceutical and nutraceutical potentials. Different parts of *Garcinia* species have been used globally as an ethnomedicine to treat several disorders. Fruits of *G. parvifolia* have been shown to contain diverse phytochemicals and display high antioxidant and promising anti-Alzheimer’s effects [9]. Hemeshkar et al. [10] reported that compound isolated from the flower and seed of *G. dulcis* contained ethnomedical value as an antioxidative, anti-viral and anti-inflammatory agent, leaves of *G. parvifolia* and fruits of *G. scortechinii* act as antibacterial, fruits of *G. brasiliensis* act as antitumours, anti-inflammation and antiarthritis, fruit rind of *G. cambogia* and *G. indica* act as anti-obesity, anticancer and antioxidant and seed of *G. kola* and *G. afzelli* act as antioxidant. Therefore, the main objective of this study was to investigate the antiproliferative properties of different parts (peel, flesh and seed) of selected *Garcinia* species namely *G. dulcis*, *G. parvifolia*, *G. nitida*, *G. mangostana* var. *mangosta* and *G. cambogia* collected in Sabah, Malaysian Borneo against estrogen receptor-positive human breast carcinoma (MCF-7) cells.

2. Materials and Method

2.1. Plant materials and sample preparation

Fruits of five *Garcinia* species were collected from selected areas around the state of Sabah, Malaysian Borneo from October to December 2012. The voucher specimens of these plants were identified and deposited in BORNEENSIS, Institute for Tropical Biology and Conservation (BORH), Universiti Malaysia Sabah, Malaysia. The fruits were cleaned and separated accordingly. The fruits were then cut into smaller piece and stored at -80 °C (Thermo Scientific) before being freeze-dried in a freeze drier (Thermo Scientific). The freeze-dried samples then were grounded into fine powder and kept in air-tight zip-lock bag and stored in a -20 °C freezer until further analysis.

2.2. Extraction

The extraction method was adapted from Rahmat et al. [11] with slight modifications using 80 % methanol. Briefly, the samples were extracted for 2 h using selected solvent at a ratio of 1:300 (w/v). The mixture was placed in a beaker wrapped with aluminium foil and agitated with an aid of orbital shaker which was set at 200 rpm at room temperature. The mixture was filtered using filter paper (Whatman No. 4) to obtain clear solution. The filtrate was subjected to vacuum rotary evaporator
(EYELA Rotary Evaporator) at 40 °C to remove methanol residue. The concentrated methanolic extract was freeze-dried to ensure the excess water was removed and crude extracts were obtained. The crude extract was diluted in dimethyl sulfoxide (DMSO) and final dilution was made in Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine (Sigma Aldrich) containing 10 % fetal bovine serum (FBS) and 1 % penicillin streptomycin (Sigma Aldrich) before assays.

2.3. Cell culture
MCF-7 cancer cell line was obtained from American Type Culture Collection (ATCC, USA). The cancer cell line was first cultured in RPMI 1640 medium with L-glutamine (Sigma Aldrich) and supplemented with 10 % of FBS, 1 % penicillin streptomycin (Sigma Aldrich) in 75 cm² flask (Falcon) and incubated at 37 °C with 5 % CO₂ in a humidified atmosphere (Shimadzu, Japan).

2.4. Cell plating
Cell growth was observed under inverted phase-contrast microscope to determine its confluences. Firstly, the old media was discarded and washed once/twice with PBS. Then, the cells were trypsinized using trypsin-EDTA for 5 min. The cells were observed under inverted phase-contrast microscope to ensure all cells were fully detached. Then, the cells were resuspended few times with media to ensure the suspension was well mixed. Then, 20 µl of cell was added into 20 µl of trypan blue for cell counting using the haemocytometer. The cells were diluted in culture medium to a density of 1 x 10⁶ cells/ml. From this cell suspension, 100 µl was pipetted into each well of a 96-well plate (Falcon, USA) and incubated for 24 h at 37 °C with 5 % CO₂ in a humidified atmosphere.

2.5. Cell treatment
After 24 h of incubation, the old medium was pipetted out from the plate. The cells were then treated with fruit extracts at doses of 1.5625, 3.1250, 6.2500, 12.5000, 25.0000, 50.0000 and highest concentration of 100.0000 µg/ml. The cells with sample extracts were then maintained at 37 °C with 5 % CO₂ for 72 h.

2.6. MTT assay
The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was based on the ability of mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and formed dark blue formazan crystals which was largely impermeable to cell membranes resulting in accumulation within healthy cells [12]. After 72 h, MTT reagent (20 µl) was added into each well and the plate was incubated for another 4 h. After that, the formazan crystals were solubilised with 100 µl of solubilization solution (DMSO) in each well. Reading was taken at 570 nm using microplate reader (ELISA Reader, Tecan), from which cytotoxicity was determined using the following formula:

\[
\text{% cytotoxicity} = \frac{\text{optical density of sample}}{\text{optical density of control}} \times 100
\]

The final results were expressed as IC₅₀ value (the concentration of sample able to inhibit cell proliferation by 50 %; µg/ml) that was calculated graphically for each cell proliferation curve.

2.7. Statistical analysis
The results for percentage of cell viability were reported as mean ± standard deviation (SD) from triplicate determinations using Statistical Package for the Social Sciences (SPSS) version 21.0. The data was statistically analyzed by one-way analysis of variance (ANOVA). The p value of less than 0.05 was considered as statistically significant.
3. Results and Discussion

MTT assay is a common method to investigate the cytotoxicity of natural products. The MTT assay is based on conversion of MTT into insoluble purple formazan by a mitochondrial enzyme in living cells. The amount of formazan produced is directly proportional to the number of viable cells [13]. In simpler terms, the changes of yellow colour to purple indicate of living cells. Table 1 shows the antiproliferative effects of five species of *Garcinia*. The results showed that all fruit parts of *G. dulcis*, peel of *G. mangostana* var. *mangosta*, peel and seed of *G. cambogia* were able to induce antiproliferative activity against MCF-7 cancer cell line. Lower IC$_{50}$ value obtained indicates stronger antiproliferative activity. *G. dulcis* seed showed the most prominent and promising result as anticancer agent with the lowest IC$_{50}$ value of 2.5 µg/ml, followed by *G. dulcis* flesh and *G. mangostana* var. *mangosta* peel but they were not differ significantly. The highest IC$_{50}$ value was detected in *G. cambogia* peel. However, none of the fruit parts of *G. parvifolia* and *G. nitida* were able to induce antiproliferative activity in MCF-7 cancer cell line at concentration less than or equal to 100 µg/ml. The inhibition of MCF-7 cancer cell lines could be partially explained by the presence of phenolic and flavonoid compounds such as xanthone that mainly distributed in *Garcinia* species.

Previous study reported that *G. dulcis* seed possessed xanthone compound namely, Dulcisxanthon G [1,3,6-trihydroxy-2-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-8-(3-methyl-2-buteryl) xanthone] together with 13 other known compounds [14]. In addition to that, isolation of compounds from *G. dulcis* ripe fruit had identified 24 compounds such as dulcisflavan, dulcisxanthon B and (-) epicatechin [15]. Besides that, kaempferol (kaempferol 3,7-di-O-α-rhamnopyranoside) was also found in *G. dulcis* ripe fruit as reported earlier by Mahabusarakam *et al.* [16] and this compound was able to inhibit the activity of hormone/estrogen dependent breast cancer cell line (MCF-7). *G. mangostana* has been widely studied and known to possess anticancer properties, contributed from the mangostin compound (a type of xanthone) especially α- and γ- mangostin [17]. The fruit pericarp of *G. mangostana* has been reported to exhibit antioxidant [18], antiproliferative, pro-apoptotic [19], anti-inflammatory [20], anti-microbial and anti-fungal [21, 22]. Recent study reported that consumption of mangosteen pericarp extract (81 % α-mangostin and 16 % of γ-mangostin) in ratio of 0.25 % and 0.5 % extract to food dosage in daily diet has been proven to inhibit tumor growth in HCT116 (human colorectal carcinoma) and reduce blood vessels in tumor towards Athymic NCr nu/nu mice as in vivo tested subject [19]. Even though the sample in this current study was from *mangosta* variety and could only be found at Agricultural Research Station, Tenom, Sabah, Malaysia, the secondary metabolites and its potential to induce antiproliferative activity ought to be the same as they were from the same genus and species.

Previous study also showed the ability of *G. cambogia* peel and seed to induce antiproliferative activity against MCF-7 cancer cell line. This might be due to high (-)-hydroxyecitrlic acid content in the fruit [23]. Besides that, xanthone compounds such as garcinol and guttiferone were also found in *G. cambogia* fruit. Hence, these compounds might contribute to antiproliferative activity of tested cancer cell line. In this study, no antiproliferative activity was observed in both *G. parvifolia* and *G. nitida* at concentration tested. Even though they were from the same genus but the capability to induce antiproliferative activity at low concentration might differ due to several factors such as phytochemical content present. According to Abu Bakar *et al.* [24], specific phytochemicals might act additively, synergistically and/or antagonistically with other compounds to display antiproliferative activity. In addition, numerous phytochemicals derived from edible plants have been reported to interfere with a specific stage of the carcinogenic process, which might act as cancer blocking and suppressing agents, preventing the carcinogenic initiation and inhibiting cancer promotion and progression [13]. Many previous studies have shown the effectiveness of edible fruit extracts as anticancer agent. For instance, cranberry, lemon, apple, strawberry, red grape, banana and grapefruit showed potent antiproliferative activity towards HepG2 cell line [25] while rowanberry, raspberry, lingonberry, cloudberry, arctic bramble, and strawberry towards HeLa (human cervical cancer) cell line [26]. Bambangan (*Mangifera pajang*) also showed potent antiproliferative activity against MCF-7 cancer cell line [27].
Table 1. Antiproliferative activities of five *Garcinia* species towards hormone-dependent breast cancer cell lines by using MTT assay

| Species                  | Parts       | MCF-7          |
|--------------------------|-------------|----------------|
| *G. dulcis*              | Peel        | 17.67 ± 2.08<sup>c</sup> |
|                          | Flesh       | 9.33 ± 3.21<sup>b</sup>  |
|                          | Seed        | 2.5 ± 0.00<sup>a</sup>   |
| *G. parvifolia*          | Peel        | ND             |
|                          | Flesh       | ND             |
|                          | Seed        | ND             |
| *G. nitida*              | Peel        | ND             |
|                          | Flesh       | ND             |
|                          | Seed        | ND             |
| *G. mangostana var. mangosta* | Peel       | 11.17 ± 1.04<sup>b</sup> |
|                          | Flesh       | ND             |
|                          | Seed        | ND             |
| *G. cambogia*            | Peel        | 56.67 ± 10.5<sup>e</sup> |
|                          | Flesh       | ND             |
|                          | Seed        | 37.67 ± 3.51<sup>d</sup> |

Data are presented in mean ± SD (n=3). Different alphabet indicate significant difference (p<0.05).

N.D = not detected in concentration less than or equal to 100 µg/ml.

4. Conclusion

*G. dulcis*, *G. mangostana var. mangosta* and *G. cambogia* fruits displayed potent antiproliferative activity against MCF-7 cancer cell line but no activity was observed in *G. parvifolia* and *G. nitida*. The results suggest that the whole fruit of *G. dulcis* should be further studied as they have the potential to be leading pharmaceutical products as anticancer agent.

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