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Research Article

Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of *Garcinia nervosa* (Clusiaceae)

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In our continuing interest on Sarawak *Garcinia* species, we carried out the evaluation of antioxidant, anti-inflammatory and cytotoxic activities on the methanolic extracts of *Garcinia nervosa*. The extracts were prepared from its air-dried grounded leaves and barks. The evaluation of antioxidant activities was done using the (2,2-diphenyl-1-picrylhydrazyl) DPPH radical scavenging assay and the result showed high radical scavenging activities. Meanwhile, the anti-inflammatory evaluation was performed using the lipoxygenase assay, hyaluronidase assay, and xanthine oxidase assay which showed, both of these extracts exhibited high anti-inflammatory properties. The lipoxygenase assay showed a high inhibition of enzyme activity for the barks extracts and a moderate enzyme activity for the leaves extracts. However, there were low inhibitions for both extracts in the hyaluronidase assay and only the barks extracts exhibited moderate antigout properties in the xanthine oxidase assay. For the cytotoxic assay, the extracts exhibited positive responses against the three cancer cell lines, the HeLa cell lines, MCF-7 cell lines, and HT-29 cell lines. Thus, *Garcinia nervosa* contains high antioxidative and anti-inflammation properties, which have great potential in the development of pharmaceutical and dermatological products.

1. Introduction

Tropical plants are beneficial and can be manipulated for therapeutic purpose due to the high content of rich biological active compounds. That will be including the *Garcinia* which is a plant genus of the family Clusiaceaenative to the "Old World" which are Asia, Australia, tropical and southern Africa, and Polynesia. There are studies which have been reported on different *Garcinia* species from various places but not *Garcini nervosa* from Sarawak. Our very own *Garcinia a nervosa* may serve as a potential source of the bioactive compounds like the other *Garcinia* species. Since there were no reports on *Garcinia nervosa*, this will be the first paper reporting on the biological activities of the *Garcinia nervosa* from Sarawak.

In accordance to the previous studies on the *Garcinia* species, *Garcinia* are used traditionally for the treatment of abdominal pain, infected wound, dysentery, diarrhoea, suppuration, leucorrhoea, chronic ulcer, and gonorrhea [1]. *Garcinia* is anti-inflammatory and anti-immunosuppresive [2]. In addition, *Garcinia* has antitumor and antioxidant abilities [3]. It was also reported that the bioactive substances that are found in *Garcinia* showed anti-inflammatory effects [4].

Garcinia species are rich sources of mangostin, tannin, xanthone, isoflavone, flavones, and other bioactive substances [5, 6]. It is proven by extensive research that some bio-compounds from *Garcinia* species exhibited a wide range of biological and pharmacological activities such as cytotoxicity and antioxidant abilities.

Previous cytotoxic studies on xanthones from *G. xipshuanbannaensis* [7] and benzoquinone from *Garcinia atroviridis* [8] exhibited significant cytotoxic activity on human cervical cancer cell lines (HeLa). Moreover, polyprenylxanthones from *G. bracteata* exhibited significant cytotoxic activity against KB cells [9] while yahyaxanthone from

Garcinia rigida leaves showed significant inhibitory to L1210 cell [10]. The xanthones of *G. penangiana* also exhibited significant cytotoxic activity on human prostate carcinoma cell line (DU-145), human breast adenocarcinoma cell line (MCF-7), and human lung cancer cell line (NCI-H460) [11]. Besides that, it was also reported that guttiferones Q-S, found in the pericarp of *G. cochinchinensis* collected in Vietnam, showed potential cytotoxicity, having IC_{50} values in the range of 2.74–4.04 mg/mL, against three human cancer cell lines, which are MCF-7, HeLa, and NCI-H460 [12].

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 [13]. Although there were a large number of studies which have been conducted on the Garcinias, there are no intensive studies and proper modern analysis of medicinal properties reported on *Garcinia nervosa* from Sarawak. Thus, it would be an interesting study on this species of indigenous tropical plant of Sarawak in order to develop a new chemical profile not only for the purpose of expanding knowledge, but also as an alternative for medicinal purpose to cure diseases.

2. Experimental Section

The leaves and barks samples of *Garcinia nervosa* were collected from local area of Kuching, Sarawak. Herbarium voucher specimens were prepared and deposited at the Herbarium of Forestry Research Centre (FRC), Sarawak Forestry Cooperation Sdn Bhd and Natural Products Research and Development Centre (NPRDC), Universiti Teknologi MARA (UiTM) Sarawak. The samples were air-dried and grinded to powder and later extracted with methanol for 24 hours at room temperature. The extracts were dried under reduced pressure using the rotavapor BUCHI model R-200. The extracts were stored at 4°C until being used.

3. Antioxidant Activity

The methanolic extracts were then evaluated for the antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl). The antioxidant activity evaluated by free radical scavenging activity which was measured by 1,2-diphenyl-2-picrylhydrazyl (DPPH). The DPPH is a stable free radical, thus the free radical reducing activity of antioxidant base on the one-electron reduction evaluated by the scavenging of DPPH. In addition, the antioxidant potential of the test sample, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system can also be determined by the scavenging of DPPH. This DPPH method was carried out according to Blois (1958) method as described by Pin et al. [14]. 200 μL of samples (1.0 mg/mL—which were prepared in triplicates) was added to 200 μL of DPPH (1 mM in ethanolic solution) and 600 μL of absolute ethanol (AR Grade) in a 10 mL amber bottle with screw cap. The mixture was shaken well and allowed to stand at room temperature for 10 minutes. Then, the absorbance at 520 nm was measured with spectrophotometer. The radical scavenging activity is described by percentage:

$$\frac{\text{Absorbance Negative contol} - \text{Absorbance Positive control}}{\text{Absorbance Negative control} - \text{Absorbance Sampel}} \times 100. \quad (1)$$

4. Anti-Inflammatory Activities

The evaluation was performed using the lipoxygenase assay [15], hyaluronidase assay [16], and xanthine oxidase assay [17] with slight modification. The stock solution of test sample for lipoxygenase and xanthine oxidase assays as prepared in methanol at concentration of 20 mg/mL before being diluted in phosphate buffer pH 8 to give concentration 2 mg/mL. Meanwhile, stock solution of test sample for hyaluronidase assay was solubilized in DMSO to prepare a concentration of 5 mg/mL. Nordihydroguaiaretic acid (NDGA), apigenin, and allopurinol were used as reference compounds for lipoxygenase, hyaluronidase and xanthine oxidase assays. Triplicate samples were prepared.

The lipoxygenase assay was performed with 160 μL of 100 mM sodium phosphate buffer (pH 8.0), 10 μL of test-compound solution and 20 μL of lipoxidase enzyme solution were mixed, dissolved in DMSO and incubated for 10 min at 25°C. The reaction only initiated when 10 μL linoleic acid (substrate) solutions was added. The test was performed under the absorbance at 234 nm. Meanwhile, for xanthine oxidase assay, 130 μL of 0.05 M potassium phosphate buffer (pH 7.5), 10 μL of test compound solution, and 10 μL xanthine oxidase enzyme were mixed, dissolved in DMSO and the reaction only took place when 100 μL xanthine (substrate) solution was added and incubated for 10 min at 25°C. The enzymatic conversion of xanthine to form uric acid and hydrogen peroxides measured at absorbance of 295 nm. For both the assay mentioned above, all the reactions were performed in triplicate, where in 96-well microplates Tecan Infinite M200 Microplate Reader for the lipoxygenase assay and 96-well UV microplate for the xanthine oxidase assay.

In the hyaluronidase assay medium, there were 80 U hyaluronidase in 100 μL 20 mM sodium phosphate buffers and 25 μL of the test samples (in DMSO) were preincubated for 10 min at 37°C. 100 ul hyaluronic acid was added and incubated again for 45 min at 37°C. The undigested hyaluronic acid was precipitated with 1 mL acid albumin solution and incubated for 10 min at room temperature before measured at the 600 nm absorbance.

5. Cytotoxic Activity

The cytotoxicity was determined by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay against HeLa and MCF-7 (human cervical cancer cell lines) and HT-29 (human colon adenocarcinoma cell lines). The assay was performed with slight modifications as described by Mosmann (1983). MTT was first prepared as a stock solution of 5 mg/mL in phosphate buffer (PBS,

Table 1: DPPH Radical scavenging activity of methanolic crude extract of *Garcinia nervosa*.

Part	DPPH Radical scavenging activity (%)
Bark	98.73
Leaves	97.40

pH 7.2) and filtered. Samples of different concentration of the extract were prepared in triplicates. At the end of the treatment period (72 h), the 20 μL of MTT solution was added to each sample and allowed to incubate for another 3 hours. During the incubation period, viable cells converted MTT to a water-insoluble formazan dye. The plates were then centrifuged (Centrifuge 5810R, Eppendorf) at 400 x g and 4°C. All remaining supernatant were then removed and 100 μL of dimethylsulphoxide (DMSO, Fisher Scientific) was added to each well. The plates were reincubated, as was done earlier, for an hour to dissolve the formed crystal formazan. The formation of the dye was proportional to the number of viable cells which was detected using a microplate spectrophotometer (μQuant Universal Microplate Spectrophotometer, BIOTEK Instrument, Inc.) at 570 nm wavelength. The mean absorbance for each compound concentration was expressed as a percentage of the absorbance of the control untreated well and plotted versus compound dose. The IC_{50} values represented the concentration that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells. The positive control for the cell lines of HeLa was cisplatin while the MCF-7 and HT-27 were goniothalamin and 5-fluorouracil respectively. The medium was used as the negative control.

6. Result and Discussion

The methanolic crude extracts from the barks and leaves of *Garcinia nervosa* were evaluated to investigate the presence antioxidant activity and its potential and effectiveness towards cell repair, rebuilding or, reproduction. The result obtained from both the barks and leaves extracts showed a very active antioxidant activity whereabouts; the barks and leaves extracts demonstrated a high percentage of scavenging activity with 98.73% and 97.40%, respectively. The results showed that *G. nervosa* is very potent for further development in producing antioxidant products since the test for DPPH radical scavenging activity gives excellent positive results. The results were tabulated in Table 1.

Garcinia nervosa extracts also exhibited high anti-inflammatory properties. In all the three assays, the final concentration of samples was 100 ug/mL. The indication for the range of inhibition 70–100% was considered as high, 40–69% was considered as moderate, and 1–39% as low inhibition and there was no inhibition or it was considered as not active at 0%.

In the lipoxygenase assay, the results showed a high inhibition of enzyme activity with 86.5% inhibition for the barks methanol extracts. Meanwhile, a moderate enzyme activity with 62.54% inhibition for the leaves methanol extracts was shown. However, when tested using the xanthine oxidase assay, the barks extracts also exhibited moderate antigout properties with 59.5% inhibition and low inhibition took place for the leaves extracts. The other assay conducted was the hyaluronidase assay where the results obtained were low inhibitions for both of the leaves and the barks extracts. All the results for the three assays were put in percentage figure which was tabulated as shown in Table 2.

In addition, the cytotoxic assay was conducted and the crude extracts were tested against HeLa, MCF-7, and HT-29 cell lines. From the results obtained, the extracts gave positive response to the cell lines especially for the leaves extracts that show high and consistent cytotoxic activity in all the three cancer cell lines. The barks extracts indicated a moderate activity and was also consistent in the three cell lines. The results obtained were tabulated in Table 3 and illustrated in Figures 1, 2 and 3.

Table 2: Anti-inflammatory activities of methanolic crude extracts of *Garcinia nervosa*.

Anti-inflammatory activities	% Inhibition	
	Barks	Leaves
Hyaluronidase assay	2.74	16.68
Lipoxygenase assay	62.54	86.53
Xanthine oxidase assay	59.50	37.09

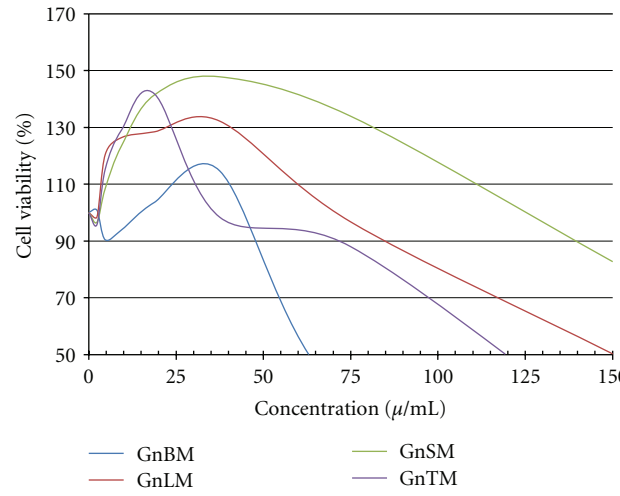

Figure 1: Cytotoxicity against HeLa cell lines.

Table 3: Inhibitory concentration of 50% by HeLa, MCF-7, and HT-29.

Independent replicate	HeLa		MCF-7		HT-29	
	1	2	1	2	1	2
GnBM	67.5	66	22.5	28	57.5	62
GnLM	149	—	125	111	116	103
GnSM	—	—	—	—	—	—
GnTM	119	118	85.5	70.5	74	78

Results were obtained from 2 replicates of 2 independent experiments.
Note: GnBM: *Garcinia nervosa* bark methanol extracts, GnLM: *Garcinia nervosa* leaf methanol extracts, GnSM: *Garcinia nervosa* stem methanol extracts, GnTM: *Garcinia nervosa* twig methanol extracts.

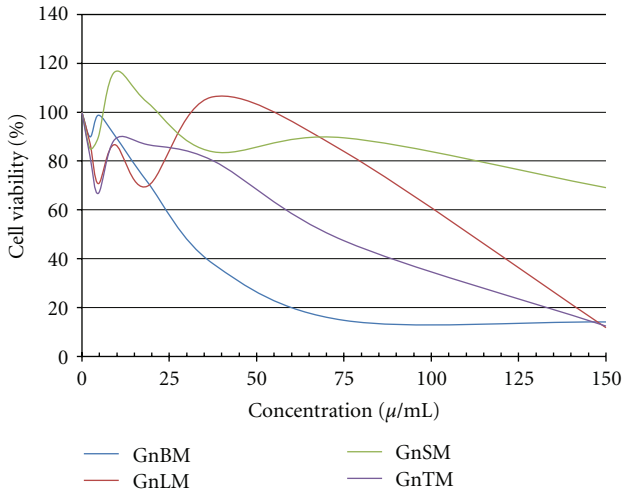

Figure 2: Cytotoxicity against MCF-7 cell lines.

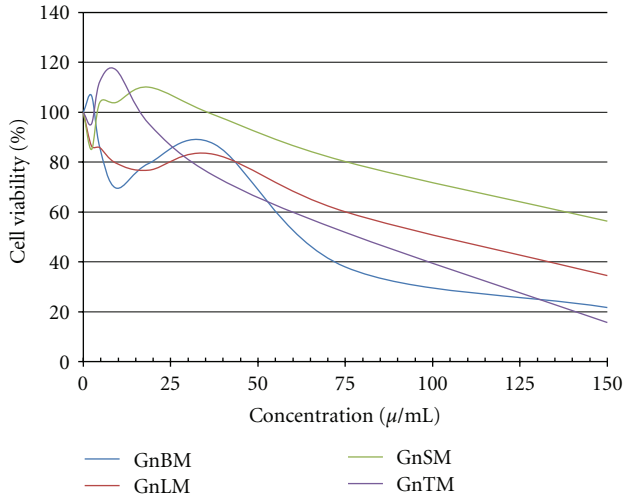

Figure 3: Cytotoxicity against HT-29 cell lines.

7. Conclusion

The bioassay studies showed that *Garcinia nervosa* exhibited high anti-oxidative and anti-inflammation properties. It also demonstrated positive responses against the three cancer cell lines, the HeLa cell lines, MCF-7 cell lines, and HT-29 cell lines. This will lead to a great potential in the development of the pharmaceutical and dermatological products in terms of the quality and the effectiveness. Thus, it is worth to proceed with further work on *Garcinia nervosa* as an afford to expand the findings of this study. The isolation and elucidation work is being carried out to identify the bioactive compounds present in this *Garcinia* species from Sarawak.

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