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Cytotoxic and Anti-Inflammatory Activities of *Garcinia xanthochymus* Extracts on Cell Lines

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Abstract

Objective: *Garcinia xanthochymus* extract has been reported to have several pharmacological properties. This study was conducted to evaluate cytotoxic and anti-inflammatory activities of *G. xanthochymus* extracts on cell lines. **Methods**: The roots and stem barks of plant were extracted using maceration method with *n*-hexane, dichloromethane and methanol, successively. Cytotoxic activity of the extracts was tested against MCF-7 breast adenocarcinoma using MTT assay. Anti-inflammatory study was evaluated using RAW 264.7 mouse macrophage cells. The nitric oxide production in LPS-stimulated cells was measured using Griess reagent. **Results**: The results of cytotoxic and anti-inflammatory study showed that dichloromethane and *n*-hexane extracts of root and stem bark exhibited cytotoxic activity in dose-dependent manner. Meanwhile, for anti-inflammatory study, all root extracts together with stem bark dichloromethane and *n*-hexane extracts reduce NO production in LPS-stimulated cells in dose dependent manner. **Conclusions**: This finding indicated that *G. xanthochymus* extracts might become interesting candidate for treatment of cancer and inflammation.

Keywords: Garcinia xanthochymus, Guttiferae, cytotoxicity, anti-inflammatory, in vitro

Introduction

Cancer is a major global burden and according to World Health Organization (WHO), 14.1 million new cases of cancer reported worldwide and 8.2 million people died due to cancer in 2012. Cancer is defined as the uncontrolled division of abnormal cells and able to invade other tissues. It may occur when a cell starts ignoring the instruction of the cell cycle checkpoint mechanism. This mechanism controls the rate of cell proliferation and as it is failed, cell eventually starts to divide in an uncontrolled manner.[1] Major concern involves the sharp rise in breast cancer cases since it is the most leading cause of cancer death and frequently diagnosed cancer among women in 140 out of 184 countries worldwide.[2] Increment in burden brings about concern to find new therapy against cancer which is cost effective with fewer side effects. Meanwhile, inflammation is the defensive response of the body which is non-specific towards the tissue damage. There are many conditions that could trigger inflammation such as pathogens, abrasions, chemical irritation, distortion or disturbances of cells and extreme temperature. A normal inflammation response should be short-lived which means it is self-limited by the down-regulations of pro-inflammatory proteins and up-regulations of anti-inflammatory proteins.[3] Even though it is a good host defensive response, prolonged inflammation can result in various physiological dysfunctions.[4] This undesirable condition leads to impairment of regular physiology and development of inflammatory diseases such as rheumatic arthritis, multiple sclerosis, inflammatory bowel disease and gastritis.[5,6]

The utilization of natural sources to search for new pharmacologically active agent has led to the discovery of many clinically beneficial drugs that contributes to a vital role in the treatment of human disease. Up till today, natural product continues to play essential roles in healthcare. In 1985, the WHO estimated that

approximately 65% of the world population depends on plant-derived traditional medicine for their primary health care meanwhile in remaining population of developed countries, plant products still play indirect role in the healthcare system.[7] *Garcinia* species is common in Southeast Asia. This species is rich source of phytochemicals that include xanthones, flavonoids, and phenolic acids.[8] Other than that, plant species originated from this genus are famously known for its medicinal values of antiprotozoal, antibacterial, anti-inflammatory, anti-immunosuppressive, anti-fungal, and anti-oxidative.[9] *G. xanthochymus* was commonly used by old folk medicine for bilious condition, diarrhea, and dysentery.[10] In vitro investigation reported antioxidant activity and cytotoxic activity against SW-480 colon cancer cells of benzophenones from the fruit of *G. xanthochymus.*[11] Other antioxidant activity also shown by leaf, root and fruits extracts from *G. xanthochymus.*[10] Therefore, it can be concluded that plant of this species have good antioxidant properties and this present study was conducted to investigate the ability of *G. xanthochymus* root and stem bark extracts in cytotoxicity and anti-inflammatory activities towards selected cell lines

Methods

Plant material and crude extract preparation. The *G. xanthochymus* root and stem bark (3.465 kg and 702.5 g) were obtained on 28th August 2013 from West Sumatera, Indonesia. Plant species was identified by Dr Shamsul Khamis from University Putra Malaysia and voucher specimen (PIIUM 0222) was deposited at herbarium of Kulliyyah of Pharmacy, International Islamic University Malaysia.

Dried and powdered root and stem bark macerated with *n*-hexane, DCM and MeOH successively for 3 times for 3 days each. The extracts were then filtered and evaporated to dryness at 50 °C using rotary evaporator to yield concentrated crude extract. Total yield (w/w) for root extracts were 0.34% for *n*-hexane extract, 0.23% for DCM extract and 4.96% for MeOH extract. Meanwhile, for stem bark extracts the total yield (w/w) were 0.21% for *n*-hexane extract, 0.92% for DCM extract and 4.54% for MeOH extract.

Cytotoxic activity. Cytotoxic activity was carried out using MTT assay as previously described[12] with modification on MCF-7 breast adenocarcinorma cells. MCF-7 cell was obtained from the courtesy of Prof. Masa-Aki Ikeda of Tokyo Medical and Dental University, Tokyo, Japan. Cells were grown in complete medium (DMEM with 10% foetal bovine serum and 1% penicillin-streptomycin) at 37 °C with 5% of CO_2. MCF-7 cells were seeded in 96-well plate at density of 2.0×10^5 cells/mL. After reached confluence, cells were treated with samples (0-526 µg/mL) dissolved in ethanol for 24 hours. After treatment ended, cells were washed with phosphate buffer saline (PBS) twice and 20 µL of MTT (Calbiochem, USA) solution (5 mg/mL) was added to each well and further incubated for 4 hours. Then, 100 µL of DMSO (Fisher Chemical, Thermo Fisher Scientific, USA) was added and the microplate was left at room temperature for 1 hour. After 1 hour, the absorbance was measured using microplate reader at 570 nm wavelength and 630 nm reference wavelength. The IC_{50} was established by extrapolation from linear regression of the data from cell viability percentage.

Anti-inflammatory activity. RAW 264.7 mouse macrophage cell was purchased from American Type Culture Collection (ATCC), Virginia, USA. RAW 264.7 macrophage cells used in this anti-inflammatory study were less than 20 passages. Cells were seeded into 96-well with density of $4.0\text{-}5.0\times10^5$ cells/mL and exposed with samples (0-100 µg/mL) for 24 hours at 37 °C with 5% of CO_2 atmosphere. After 24 hours, cell viability was assessed using MTT assay as described previously.

Nitric oxide (NO) production. NO production determination was done as previously described[13] with minor modification. RAW 264.7 macrophage cells were seeded into 24-well plate at density of $5.0\text{-}6.0\times10^5$ cells/mL until it reached confluent. Cells were pre-treated with or without samples (10-60 µg/mL) for 1 hour and incubated with lipopolysaccharide (LPS) (Sigma-Aldrich Chemical Co., St. Louis, USA) at concentration of 1 µg/mL for 15 hours at 37 °C with 5% of CO_2 atmosphere. Then, 50 µL of each medium supernatant was mixed with 50 µL Griess reagent (1:1) (Sigma-Aldrich Chemical Co., St. Louis, USA) and left at room temperature for 10 minutes. Absorbance was measured at 540 nm wavelength and values obtained were compared with the $NaNO_2$ reference curve to determine the nitrite production.

Statistical analysis. All qualitative data are representative of at least three independent experiments. Values are expressed as mean±SD. Statistical analyses were conducted using one-way Analysis of Variance (ANOVA). The statistical package IBM SPSS Statistics 21 for Windows was used in the analysis.

Results

The results for cytotoxic activity of stem bark and root extracts are shown in Figure 1 and Figure 2, respectively.

The IC_{50} of the stem bark and root extracts are presented in Table 1. The stem bark and root extracts were also tested for their anti-inflammatory activity. The results for NO production inhibition are shown in Figure 3 and Figure 4.

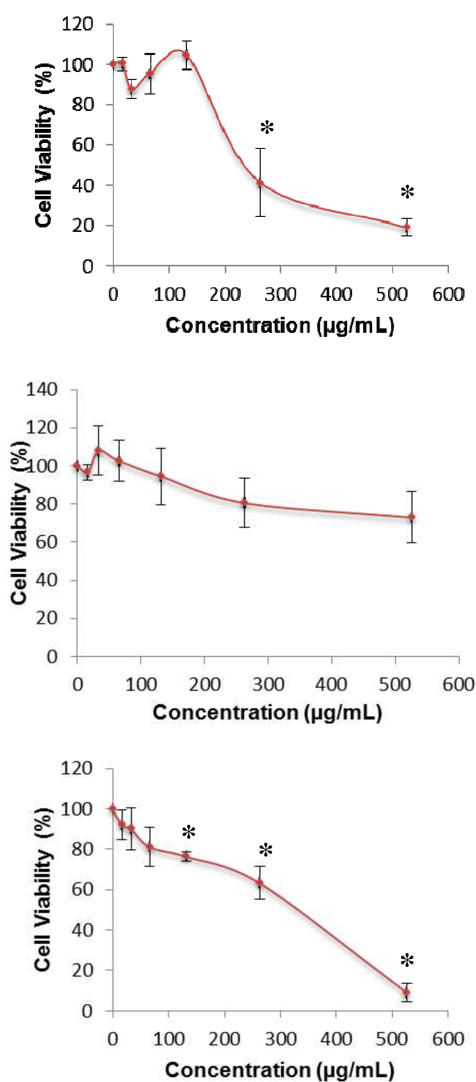

Figure 1. Effects of *G. xanthochymus* Stem Bark Extracts on MCF-7 Cell Viability. Error Bars Represent mean±SD of Cell Viability Percentage as Determined by Three Repeated Experiments. **p* < 0.05 when Compared to the Untreated Control. a) Stem Bark DCM Extract; b) Stem Bark MeOH Extract; c) Stem Bark *n*-hexane Extract

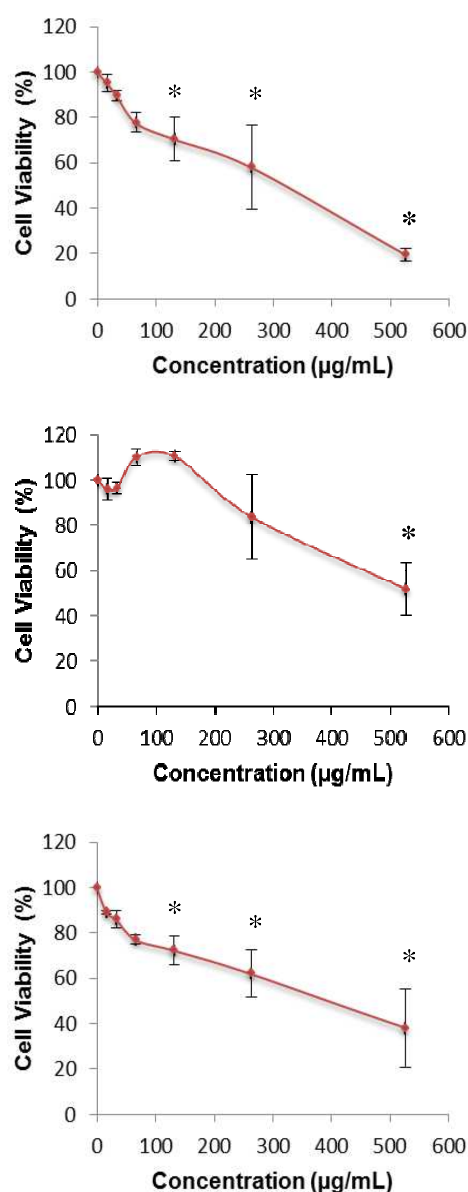

Figure 2. Effects of *G. xanthochymus* Root Extracts on MCF-7 Cell Viability. Error Bars Represent mean±SD of Cell Viability Percentage as Determined by Three Repeated Experiments. **p* < 0.05 when Compared to the Untreated Control. a) Root DCM Extract; b) Root MeOH Extract; c) Root *n*-hexane Extract

Discussion

The extracts showed cytotoxic activity in dose-dependent manner. Significant difference was observed at selected treatment concentrations when compared with untreated control. Cell without any treatment exposure was the untreated control and based from result, it showed highest cell viability. According to US NCI plant screening program, crude extract is considered cytotoxic when IC_{50} is lower than 30 µg/mL.[14] Since IC_{50} for DCM and *n*-hexane stem bark and root extracts obtained (Table 1) were more than 30 µg/mL, it can be concluded that DCM and *n*-hexane stem bark and root extracts pose

Table 1. IC_{50} of *G. xanthochymus* Extracts

G. xanthochymus extracts	IC50 (µg/mL)	Cytotoxic activity
Stem bark DCM	318.85	+
Stem bark MeOH	-	-
Stem bark n-hexane	293.51	+
Root DCM	307.30	+
Root MeOH	-	-
Root n-hexane	388.88	+

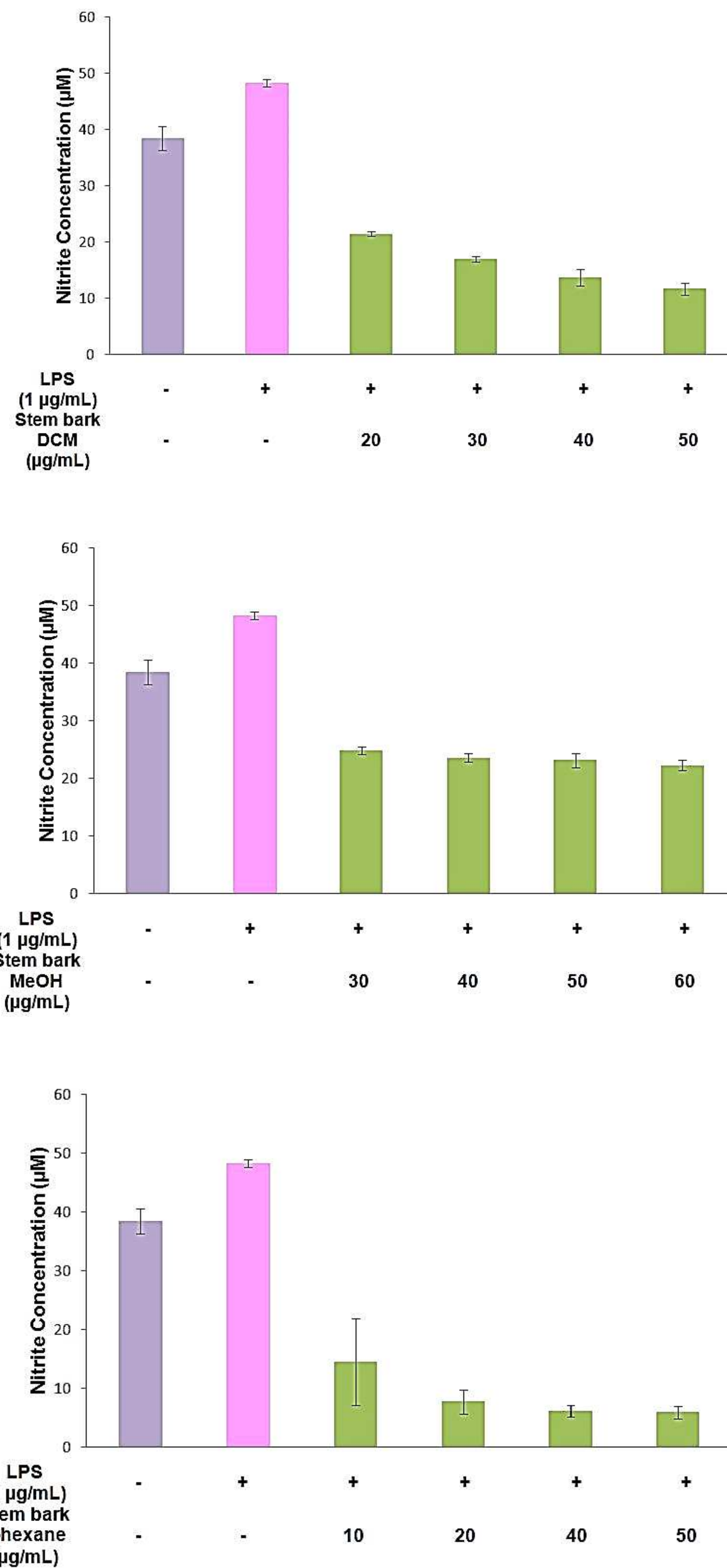

Figure 3. Effects of *G. xanthochymus* Stem Barks Extracts on NO Production Inhibition. Error Bars Represent mean±SD of Nitrite Concentration as Determined by Triplicate of Single Experiment. *$p < 0.05$ when Compared to the Positive Control. a) Stem Bark DCM Extract; b) Stem Bark MeOH Extract; c) Stem Bark *n*-hexane Extract

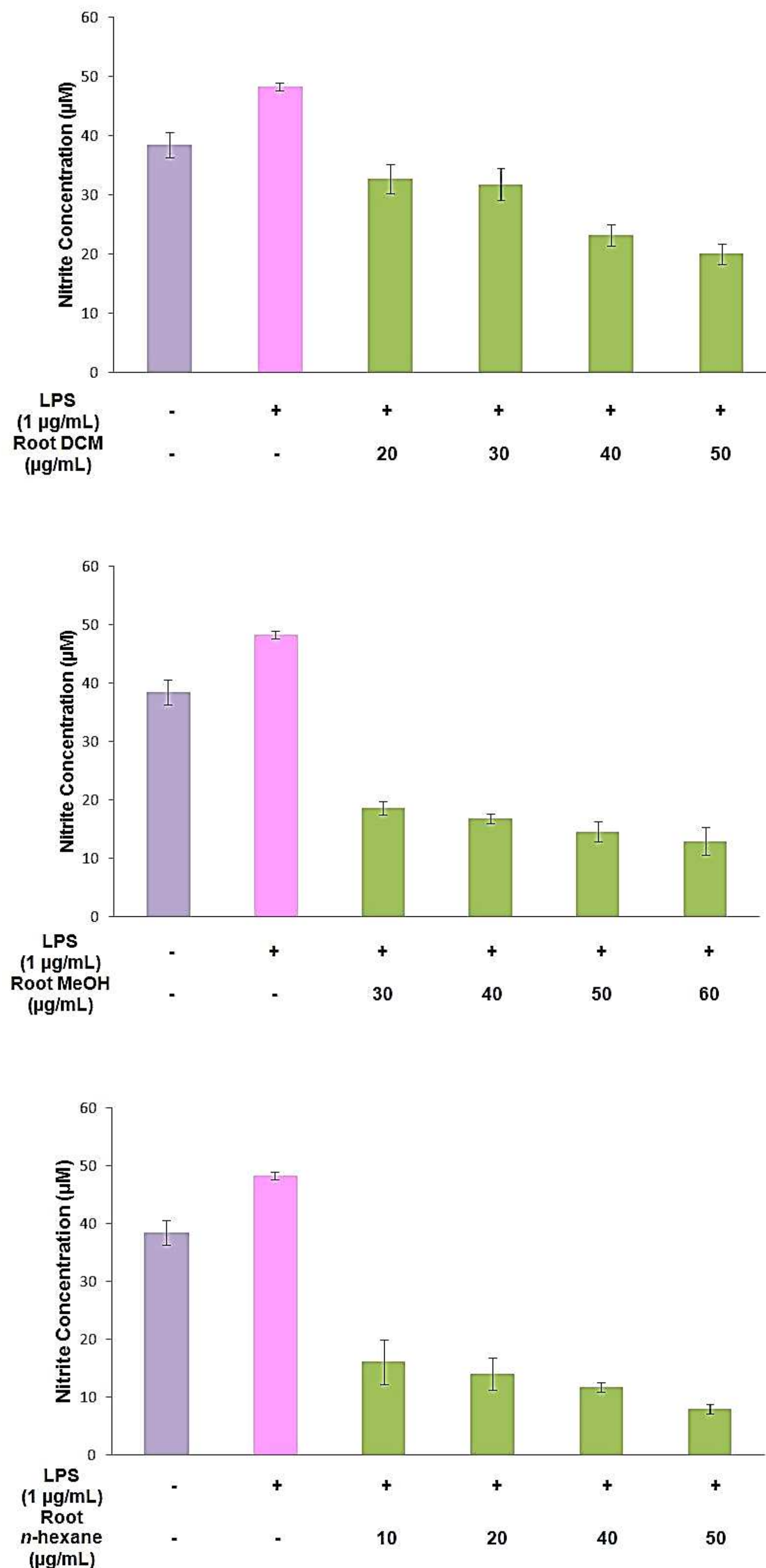

Figure 4. Effects of *G. xanthochymus* Root Extracts on NO Production Inhibition. Error Bars Represent mean±SD of Nitrite Concentration as Determined by Triplicate of Single Experiment. *$p < 0.05$ when Compared to the Positive Control. a) Root DCM Extract; b) Root MeOH Extract; c) Root *n*-hexane Extract

weak cytotoxic activity to be considered as potential anti-cancer agent. However, in terms of cytotoxicity against MCF-7 cells, IC_{50} for stem bark *n*-hexane extract was the lowest (293.51 µg/mL) as compared to the rest. Similiarly, previous investigation of *Garcinia* extracts of different species did exhibit selective cytotoxic activity on MCF-7 cell.[15]

Another aspect needed to be considered that this present study is carried out based on crude DCM, MeOH and *n*-hexane extracts. There is possibility of variety compounds exist in the plant extract and the mixture of compounds and impurities in extracts might hide the potential anti-cancer activity.

The investigation of drug interaction in multicompound remedies is difficult because of interaction among compound where some may show less specific activity and some may reduce the toxicity of more therapeutically effective plant.[16] Thus, the effect of potential anti-cancer compound can be fractionated and isolated from the crude extracts.

In order to evaluate extracts effect on anti-inflammatory activity, extracts were tested against RAW 264.7 macrophage cells. The cell viability assay was first done to determine suitable dose to be used for NO production test and possible cytotoxic effect of extracts on RAW 264.7 cells. From the cell viability result, *n*-hexane stem bark and root extracts affect cell viability with IC_{50} of 65.92 and 76.44 µg/mL respectively (data not shown). Hence, dose ranging from 10-60 µg/mL was chosen as dose for nitric oxide production test. The extracts effect on NO production inhibition was analysed using LPS-stimulated RAW 264.7 cells. Cellular model of LPS stimulated macrophage is extensively used to measure anti-inflammatory activity.[4] This study compares NO production of LPS-stimulated cell treated with extracts with positive control and negative control. Positive control used was cell exposed to LPS only. Since there is no treatment towards the cell, it was expected that the positive control produce the highest NO production. Meanwhile, negative control of the study was untreated cell without LPS induction. Untreated cell was expected to produce lowest NO since there was no exposure to inflammatory stimulus. The extracts significantly reduce nitrite oxide production in LPS-induced cells in dose-dependent manner except for stem bark MeOH extract since the extract did not shows significant decrease in NO level. Previous finding did also reported the NO reduction ability of different extract of Garcinia species on LPS and IFN-γ-induced RAW 264.7 cells.[15]

In host defence against extracellular stimuli, macrophage plays vital role. It is the first cellular mediators of the innate immune response alongside with neutrophils and dendritic cells.[17] One of the extracellular stimuli that could activate macrophage is LPS. Upon macrophage activation by LPS, the innate response of host immune response results in production of pro-inflammatory cytokines, nitric oxide (NO), and prostaglandin-E_2 (PGE_2), which are the main cytotoxic and pro-apoptotic mechanisms participating in the innate response in many mammals.[18-20] Inflammation initiated by LPS upregulates inducible nitric oxide synthase (iNOS) which later generates high NO.[13] Owing to this, measurement of NO production is a method for evaluating the anti-inflammatory effects of plant extracts.[21] Exposure of LPS to RAW 264.7 macrophage cells upregulate iNOS and pre-treatment of cell with varying extracts concentration (10-60 µg/mL) prior to LPS induction was able to reduce the NO production.

Other than that, it is interesting to observe the DCM and *n*-hexane extracts for their cytotoxicity and anti-inflammatory pattern. Good cytotoxic and anti-inflammatory activities are shown by DCM and *n*-hexane extracts of stem bark and root. Hence, further analysis can be suggested for DCM and n-hexane extracts of stem bark and root since cancer and inflammation are related in way that the inflammation responses contribute role in tumour development.[22] It is also interesting to suggest further study on DCM and *n*-hexane extract synergistic effect in reducing inflammation and killing cancer cell since such speculation can be made based on our present findings.

Conclusions

G. xanthochymus root and stem bark extracts showed dose-dependent cytotoxic activity towards MCF-7 cell line. The extracts were also able to inhibit NO production LPS-stimulated RAW 264.7 macrophage cells indicated its potential as a source for inflammation treatment.

Conflicts of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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