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Artikel Asli/*Original Articles*

Evaluation of Antiinflammatory, Antioxidant and Antiproliferative Activities of *Quassia borneensis* Noot. (Simaroubaceae) Extracts

(Penilaian Aktiviti Antiinflamasi, Antioksidan dan Antiproliferasi Ekstrak *Quassia borneensis* Noot. (Simaroubaceae)

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ABSTRACT

Quassia borneensis has been traditionally used as antihypertensive agent without any scientific literature on its mechanism of action. The objective of this study was to evaluate the antiinflammatory, antioxidant and antiproliferation properties of Q. borneensis extracts. The hexane, chloroform and aqueous extracts of root and bark of Q. borneensis were subjected to nitric oxide (NO) inhibition assay in LPS-stimulated RAW 264.7 cells. Expression of inducible NO synthase (iNOS) protein level was analyzed by Western blot. The antioxidant and antiproliferative activities of the extracts on HL-60 cells were determined using Ferric Reducing Antioxidant Power (FRAP) and MTT assays, respectively. The chloroform extract of Q. borneensis root obtained by soxhlet method (CSR) significantly inhibited 97.64 ± 0.96% of NO production ($p < 0.001$) and suppressed iNOS expression ($p < 0.05$) at the highest concentration of 1.0 µg/ml. The chloroform extract of bark obtained by maceration (CMB) exhibited the highest antioxidant capacity in the absence and presence of HL-60 cells, where the FRAP value were 125.45 ± 9.10 µM $FeSO_4.7H_2O$ and 181.55 ± 3.45 µM $FeSO_4.7H_2O$, respectively. The greatest inhibition of HL-60 cell proliferation was exhibited by the chloroform extract of bark obtained by soxhlet method (CSB) with the IC_{50} of 5.0 µg/ml. The findings suggested that the chloroform extracts of Q. borneensis possess antiinflammatory, antioxidant and antiproliferative activities.

Keywords: Quassia; Quassia borneensis; antiinflammation; *antioxidant; antiproliferation*

ABSTRAK

Quassia borneensis telah digunakan secara tradisional sebagai agen antihipertensi tanpa sebarang kajian saintifik terhadap mekanisme tindakannya. Objektif kajian ini adalah untuk menilai kandungan antiinflamasi, antioksidan dan antiproliferasi ekstrak Q. borneensis. Ekstrak heksana, kloroform dan akueus akar dan kulit Q. borneensis dinilai terhadap asai perencatan nitrik oksida (NO) dalam sel RAW 264.7 terangsang LPS. Tahap pengekspresan protein inducible NO synthase (iNOS) dianalisa dengan pemendapan Western. Aktiviti antioksidan dan antiproliferasi ekstrak terhadap sel HL-60 ditentukan dengan asai Ferric Reducing Antioxidant Power (FRAP) dan MTT, masing-masing. Ekstrak kloroform akar dengan kaedah soxhlet (CSR) secara signifikan merencat 97.64 ± 0.96% penghasilan NO ($p < 0.001$) dan menindas ekspresi iNOS ($p < 0.05$) pada kepekatan tertinggi yang diuji iaitu 1.0 µg/ml. Ekstrak kloroform kulit dengan kaedah maserasi (CMB) mempamerkan kapasiti antioksidan tertinggi dengan ketiadaan dan kehadiran sel HL-60, dimana nilai FRAP adalah sebanyak 125.45 ± 9.10 µM $FeSO_4.7H_2O$ dan 181.55 ± 3.45 µM $FeSO_4.7H_2O$, masing-masing. Perencatan terbaik terhadap proliferasi sel HL-60 dipamerkan oleh ekstrak kloroform kulit dengan kaedah soxhlet (CSB) dengan nilai IC_{50} 5.0 µg/ml. Dapatan kajian mencadangkan bahawa ekstrak kloroform Q. borneensis mempunyai aktiviti antiinflamasi, antioksidan dan antiproliferasi.

Kata kunci: Quassia; Quassia borneensis; antiinflamasi; antioksidan; antiproliferasi

INTRODUCTION

One of the strategies that contributes to a high rate of success in finding new drug candidates is through ethnopharmacological survey (Fabricant & Farnsworth 2001). For example, *Quassia amara* from *Simaroubaceae* family which is traditionally used as a bitter tonic, to treat fever and ulcers by the natives of South America was discovered to contain several bioactive compounds known as quassinoids and had scientifically shown various potent biological activities including anticancer and antimalarial properties (Bertani et al. 2006; Houel et al. 2009).

Chemical and biological research on various genera of the *Simaroubaceae* plant family such as *Ailanthus*, *Brucea*, *Eurycoma*, *Simaba*, *Quassia* and others have also been performed and the biological activities exerted by

these plant extracts were found to be contributed by the presence of a group of compounds known as quassinoid (Bhat & Karim 2010; Kundu & Laskar 2010; Lau et al. 2005; Muhammad et al. 2004). In Sabah and Sarawak of East Malaysia, an indigenous plant identified as *Quassia borneensis* Noot. has been used traditionally to treat hypertension (Goh et al. 1995). The plant can be found in primary mixed dipterocarp forest and locally called as mamungal or pait-pait (Kulip & Wong 1995). However, scientific study has not been performed on this plant to discover its potential biological activities except for the antiplasmodial activity which was recently reported (Ghazali et al. 2013; Wan Razali et al. 2015).

Realizing the huge potentials of medicinal plants from the *Simaroubaceae* family for cancer prevention and treatment due to the presence of quassinoids, hence this study was focused on three inhibitory effects on cancer development. An excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) at the site of chronic inflammation can lead to cancer. It was suggested that massive release of nitric oxide (NO) by macrophages and chemical reactions of NO with other radicals can generate oxidative and nitrosative stress. This condition will not only cause macromolecular and cellular damages, but also activate transcription factors such as nuclear factor-κB and thus promote tumor development through the regulation of cellular proliferation (Mantovani et al. 2008; Reuter et al. 2010). Therefore, these mechanisms are important targets for both cancer prevention and treatment.

The objective of this study was to evaluate the antiinflammatory, antioxidant and antiproliferative properties of *Q. borneensis* bark and root extracts. The findings of biological activities of this plant were aimed to scientifically document the benefits of its traditional application, which later will lead to the discovery of potential chemopreventive agent or chemotherapeutic drug candidate.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Lipopolysaccharides (LPS) from *Escherichia coli* 055:B5, positive control drugs and most chemicals used were purchased from Sigma-Aldrich (USA). Sodium nitrite and 2, 4, 6-Tripyridyl-s-triazine (TPTZ) were purchased from Merck (USA). Ferric chloride ($FeCl_3$) was from HmBG Chemicals (Germany) and dimethyl sulfoxide (DMSO) was from Ajax Finechem (Australia). Primary and secondary antibodies were purchased from Cell Signaling Technology (USA).

PLANT'S SAMPLING AND EXTRACTION

Samples of the plant were acquired from a fully grown tree and identified by botanist of Forest Research Centre (FRC), Sepilok, Sandakan, Sabah, Malaysia. Herbarium specimen (SAN 152508) was prepared and deposited in the FRC Herbarium. Plant samples were classified to twigs, wood, leave, root and bark before they were processed into powder form and stored in a −15°C chill room.

All chemicals used in plant extraction were of analytical grade from Merck (Germany). About 1 kg of powder form plant samples were defatted in petroleum ether and macerated in methanol for two days before concentrated *in vacuo.* The concentrated methanol extracts were suspended in 10% (v/v) aqueous methanol and partitioned between *n*-hexane and chloroform. The pooled extracts of hexane and chloroform were concentrated *in vacuo* producing hexane and chloroform extracts. The aqueous portions were freeze-dried to acquire aqueous extracts. These extracts were labeled as *Q. borneensis* macerated extracts. Another batch of extraction was carried out in soxhlet apparatus glassware for 24 h and the extracting procedure was followed accordingly. These hexane, chloroform and aqueous extracts were labeled as *Q. borneensis* soxhlet extracts. The properties of extracts produced were summarized in Table 1.

TABLE 1. Properties of *Q. borneensis* extracts tested and its antiproliferative activities on HL-60 cells after 48 h of treatment

Sample	Method	Part	Abbreviation	IC_{50} (μg/ml)
Hexane extracts	Maceration	Bark	HMB	>100
	Maceration	Root	HMR	>100
	Soxhlet	Bark	HSB	42
	Soxhlet	Root	HSR	24
Chloroform extracts	Maceration	Bark	CMB	7
	Maceration	Root	CMR	9
	Soxhlet	Bark	CSB	5
	Soxhlet	Root	CSR	6
Aqueous extracts	Maceration	Bark	AMB	6
	Maceration	Root	AMR	>100
	Soxhlet	Bark	ASB	>100
	Soxhlet	Root	ASR	19

CELL CULTURE

Human promyelocytic leukemia HL-60 (ATCC® CCL-240™) and murine monocytic macrophage RAW 264.7 (ATCC® TIB-71™) cells were obtained from American Type Culture Collection (Rockville, USA). HL-60 cells were grown in Iscove's Modified Dulbecco's Medium (Sigma, USA) with 20% (v/v) fetal bovine serum (FBS) (JR Scientific, USA). RAW 26.7 cells were grown in Dulbecco's Modified Eagle Medium (Gibco, Invitrogen, USA) with 10% FBS. Both culture media were supplemented with 1% (v/v) penicillin/streptomycin (PAA, Austria) and cells were maintained at 37 °C in 5% CO_2. The number of viable cells was determined by the trypan blue dye exclusion with a hemocytometer.

DETERMINATION OF NITRIC OXIDE PRODUCTION

Murine macrophage RAW 264.7 cells (1×10^5 cells/ml) were seeded in 96-well plate overnight and treated with 0.125−1.0 µg/ml *Q. borneensis* extracts for 2 h before LPS (1 µg/ml) was added. After 24 h treatment with *Q. borneensis* extracts at 37°C and 5% CO_2, production of nitrite, the stable conversion product of nitric oxide was determined by Griess assay (Green et al. 1982). Briefly, 100 µl of culture media was transferred to another 96-well plate and mixed with 100 µl of Griess reagent (1% sulfanilamide, 0.1% naphthalenediamine dihydrochloride) and incubated for 10 min at room temperature in dark. Color development was read at 570 nm by microplate reader (Bio-Rad, USA). Sodium nitrite (0–100 µM) was used to generate the standard curve. Indomethacin, the non-steroidal antiinflammatory drug was used as positive control. Cytotoxic effect of the extracts on RAW 264.7 cells was determined using MTT assay (Mosmann 1983) before the experiment conducted.

WESTERN BLOT ANALYSIS

RAW 264.7 (1×10^5 cells/ml) cells were treated with CSR chloroform extract at concentration of 0.125−1.0 µg/ml in 100 mm culture dish for 2 h and then stimulated with LPS (1 µg/ml). After 24 h incubation, cells were harvested and lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, phosphatase and protease inhibitors). After boiling for 5 min, the protein samples (20 µg) were separated by SDS-PAGE on 12% resolving gel and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% skimmed-milk for 1 h and then incubated with iNOS antibody (1:5000) or β-actin antibody (1:10000) overnight at 4°C. Primary antibodies were removed by washing the membranes three times in TBST and then the membranes were incubated for 1 h with horseradish peroxidase conjugated secondary antibody (Xie 2001). Detection was performed using enhanced chemiluminescence reagent (Amersham™ ECL™ Select, GE Healthcare, UK) and images were analyzed using Fusion-Capt Advance (Fusion FX7, Vilber Lourmat, Germany).

MEASUREMENT OF ANTIOXIDANT CAPACITY

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out to determine the antioxidant capacity of various extracts of *Q. borneensis* with slight modifications from Benzie & Strain (1996). The assay was performed in the presence and absence of cells as described by Hasiah et al. (2011). Concentration of *Q. borneensis* extracts tested was 6.25–100 µg/ml. In the presence of cells, HL-60 cells (2×10^5 cells/ml) were seeded in 96-well plate and treated with various concentrations of extract at 37°C and 5% CO_2. After 2 h incubation, the plate was sonicated for 30 seconds. Then, freshly prepared FRAP reagent (300 mM acetate buffer, pH 3.6, 20 mM $FeCl_3$, 10 mM TPTZ) was added and the plate was incubated for 5 min in dark. The absorbance at 595 nm was measured by microplate reader (Bio-Rad, USA). $FeSO_4.7H_2O$ solution (100–1000 µM) was used to generate calibration curve and 100 µM L-ascorbic acid was used as positive control. The antioxidant capacity of the sample was calculated using the $FeSO_4 \cdot 7H_2O$ calibration curve and expressed as FRAP value (µM $FeSO_4 \cdot 7H_2O$ equivalent).

CELL PROLIFERATION ASSAY

Antiproliferative activity of *Q. borneensis* towards HL-60 cells was determined from its viability percentage by MTT assay (Mosmann 1983). The concentration of *Q. borneensis* extracts tested was 6.25–100 µg/ml. Briefly, HL-60 cells (2×10^5 cells/ml) were seeded and treated with various concentrations of *Q. borneensis* extracts for 48 h at 37°C in 5% CO_2. Then, 20 µl of freshly prepared MTT (3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide) solution (5 mg/ml) was added into each well in dark. The culture plate was then incubated for another 4 h and the formazan crystal formed was dissolved with DMSO. The plate was incubated for 15 min and shaken for 5 min using vortex mixture. Absorbance at 570 nm was measured using microplate reader (Bio-Rad, USA). Etoposide was used as positive control. The percentage of cell viability was calculated by dividing the sample absorbance with negative control absorbance and multiplied by 100%.

STATISTICAL ANALYSIS

The results were expressed in mean ± SD from three independent experiments. Graphs were constructed with SEM error bars. The data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20. Differences in mean values between groups were analyzed by one-way ANOVA followed by post-hoc test. Statistical significant was considered at $p < 0.05$.

RESULTS

ANTIINFLAMMATORY ACTIVITIES OF *Q. borneensis* EXTRACTS

Quassia borneensis hexane, chloroform and aqueous extracts showed different degrees of cytotoxicity to RAW 264.7 cells. For the screening purpose, the highest treatment concentration of 1 µg/ml was chosen for all extracts because it was able to maintain more than 85% of cell viability after 24 h incubation as measured by MTT assay. Concentration of NO_2^- in culture medium was converted into percentage over control (LPS only).

All the hexane, chloroform and aqueous extracts of *Q. borneensis* showed significant antiinflammatory activity following 2 h pretreatment before LPS induction except CMR and ASB extracts (Figure 1). The best inhibitory effects of NO production in LPS-stimulated RAW 264.7 cells was shown by CSR extract which significantly reduced 97.64 ± 0.96% of NO production. Good antiinflammatory activity was also shown by CMB and CSB extracts which inhibited NO by 51.16 ± 2.01 and 61.02 ± 6.58%, respectively. Among hexane extracts, HMB and HSB extracts showed a marked inhibitory effect with 34.82 ± 8.54 and 32.55 ± 10.2% reduction of NO production, respectively. Other extracts give less than 25% of NO inhibition. Indomethacin as a positive control significantly inhibited the NO production by 45.12 ± 2.67%.

When tested at several concentrations ranging from 0.125 to 1.0 µg/ml, CSR extract showed dose-dependent inhibitory effect on NO production (Figure 2). Western blot analysis demonstrated that the extract was able to suppress iNOS expression at higher concentration (Figure 3). A slight increase in iNOS expression was detected at lower treatment concentration of CSR extract, but was not significantly different compared to the negative control ($p > 0.05$).

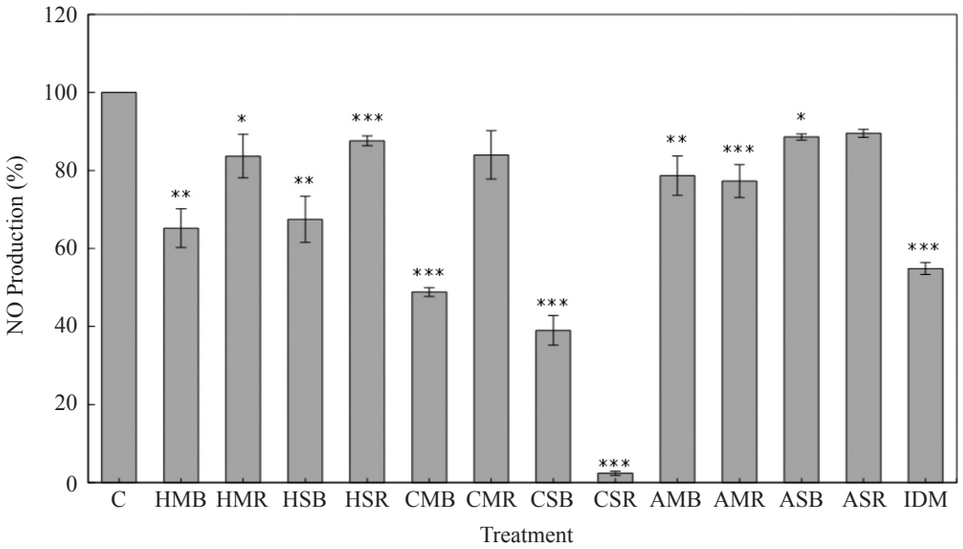

FIGURE 1. Effect of *Q. borneensis* extracts on NO production in LPS-stimulated RAW 264.7 macrophages for 24 h. Cells were treated with extracts at 1 µg/ml and Indomethacin (IDM) at 100 µM. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control (C). Abbreviation of extracts–H: hexane; C: chloroform; A: aqueous; M: maceration; S: soxhlet; B: bark; R: root

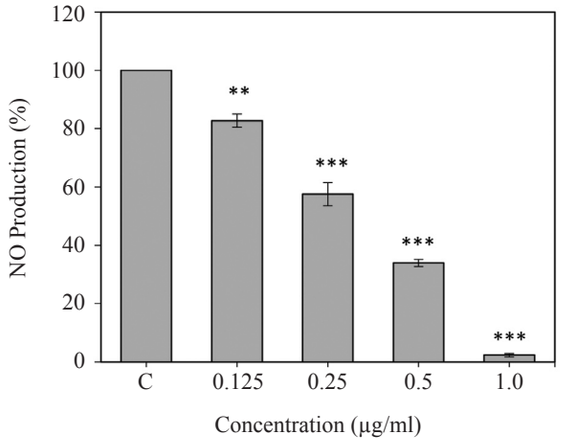

FIGURE 2. Effect of the *Q. borneensis* chloroform soxhlet root (CSR) extract on NO production in LPS-stimulated RAW 264.7 macrophages for 24 h. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control (C)

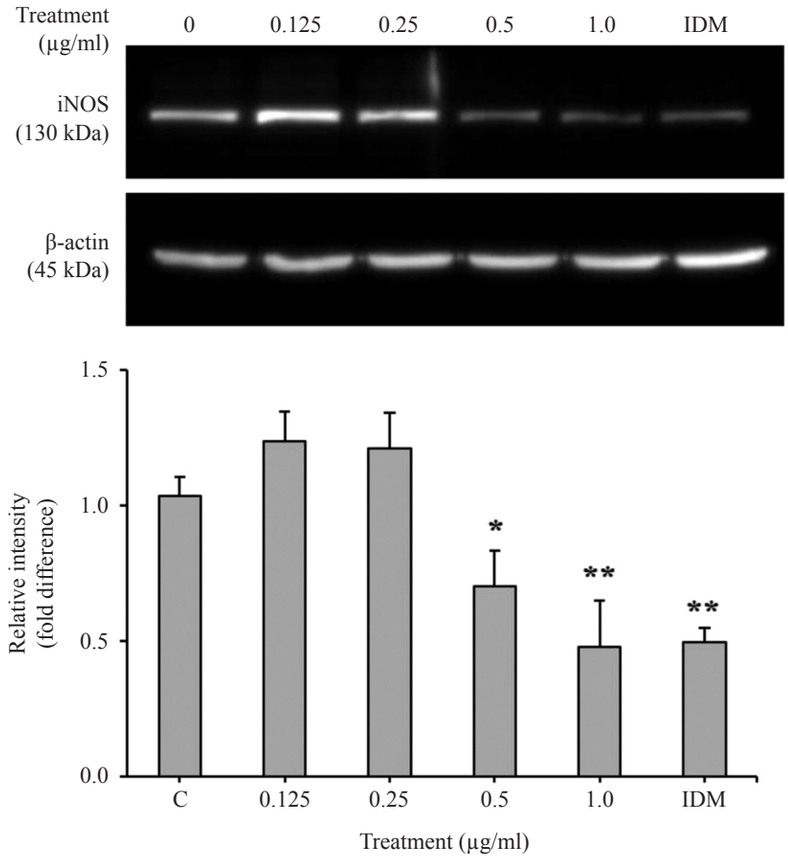

FIGURE 3. Effect of chloroform soxhlet root (CSR) extract of *Q. borneensis* and Indomethacin (IDM) (100 μM) on (a) iNOS protein expression in LPS-stimulated RAW 264.7 macrophages from Western blot analysis (top) and fold difference in relative iNOS protein band intensities that were quantified by densitometry (bottom). *p < 0.05; **p < 0.01 as compared to control (C)

ANTIOXIDANT ACTIVITIES OF *Q. borneensis* EXTRACTS

The antioxidant activity of *Q. borneensis* extracts were determined by FRAP assay which measures the ability of a substance to reduce ferric to ferrous ions. The assay was performed in two conditions; extracts only and extracts incubated with cells. Chloroform extracts demonstrated high antioxidant capacity, followed by aqueous extracts (Figure 4). The highest FRAP value obtained was given by the CMB extract (181.55 ± 5.98), while the lowest value was given by HMB extract (4.20 ± 0.07). On the other hand, untreated HL-60 cells only produce FRAP value of 0.62 ± 1.32 μM $FeSO_4 \cdot 7H_2O$. After 2 h incubation with *Q. borneensis* extracts, a significant increase ($p < 0.05$) was observed in the antioxidant capacity for all extracts treatment. Although hexane extracts alone showed low antioxidant activity, but HMB, HMR, HSB and HSR extracts were able to increase cellular antioxidant value with 7.6, 6.2, 10.9 and 4.1 times higher, respectively, when incubated with HL-60 cells. Treatment with AMR aqueous extract also showed 6.7 times significant increase of cellular antioxidant activity of HL-60 cells.

ANTIPROLIFERATIVE ACTIVITIES OF *Q. borneensis* EXTRACTS

After 48 h of treatment, the IC_{50} value which corresponded to the concentration of test substance resulting in a 50% inhibition of cell growth, was determined from the graph of percentage of cell viability versus concentration. All chloroform extracts exhibited the greatest degree of antiproliferative activity on HL-60 cells with the IC_{50} value < 10 μg/ml (Table 1). Surprisingly, AMB aqueous extract also demonstrated a significant activity as chloroform extracts. In addition, ASR aqueous extract showed better inhibition of HL-60 cell proliferation than HSR and HSB hexane extracts based on the IC_{50} value obtained. Other extracts were less effective because no IC_{50} recorded within the range of treatment concentration. Etoposide was used as positive control and inhibited HL-60 cell proliferation with IC_{50} value of 2.4 μg/ml (4 μM).

DISCUSSION

Inhibition of NO production and suppression of its molecular pathway is one of the target mechanisms for cancer prevention (Hofseth 2008). In the study, the hexane,

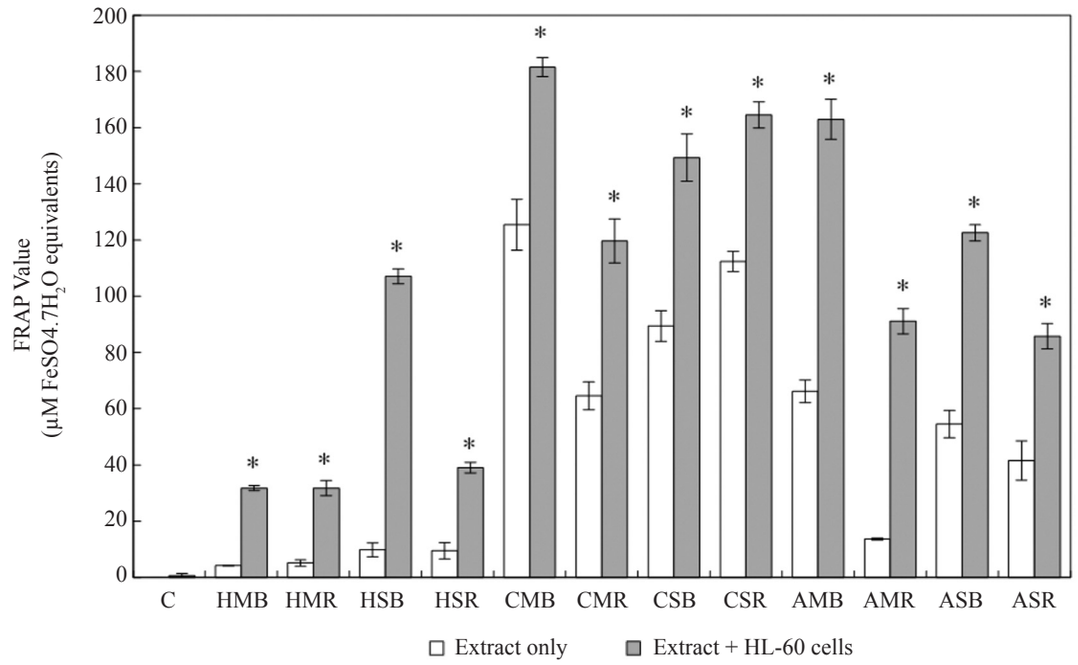

FIGURE 4. Antioxidant capacity of *Q. borneensis* extracts alone and in the presence of HL-60 cells at concentration 100 μg/ml. *p < 0.05 represent significant different of FRAP value extract + HL-60 cells compared to extract only. Abbreviation of extracts–H: hexane; C: chloroform; A: aqueous; M: maceration; S: soxhlet; B: bark; R: root

chloroform and aqueous extracts from bark and root of *Q. borneensis* were screened for NO inhibitory effects and the most prominent activity was shown by chloroform extract of *Q. borneensis* root obtained by the Soxhlet method. During the inflammatory responses, NO production was mediated by the inducible nitric oxide synthase (iNOS). This enzyme was not constitutively expressed under physiological condition, but can be stimulated by pro-inflammatory stimuli such as LPS, TNF-α and IFN-γ (Masini et al. 2010). It was evident from our results that inhibition of NO production in macrophage by *Q. borneensis* chloroform root extract was also due to the suppression of iNOS protein expression.

Several plants from Simaroubaceae family have been shown to have anti-inflammatory properties. For example, *Q. amara* extracts showed significant inhibition of iNOS and COX-2 protein through prevention of nuclear translocation of NF-κB (Verma et al. 2009). Studies on chemical constituent has identified that few quassinoids such as bruceines B and bruceines E from *B. javanica* (Liu et al. 2012) and ailanthone from *A. altissima* (Kim et al. 2015) displayed an active inhibitory effects on NO production. However, β-carbolines alkaloids isolated from *P. quassioides* were contributed to the antiinflammatory activities of the plant extract (Jiao et al. 2011). Therefore, chemical studies on is needed to elucidate the potential constituents that give the antiinflammarory effects of *Q. borneensis* extracts.

The *Q. borneensis* extracts also showed antioxidant activities by two possible mechanisms. Direct measurement of the extracts' antioxidant capacity indicated the chemical structure and reaction of an antioxidant. On the contrary, co-incubation of the extracts with cells represented the biological activity of an antioxidant. The use of cell-based assay was introduced by Wolfe & Liu (2007) using dichlorofluorescin oxidation to measure cellular antioxidant activity. However, measurement of Fe^{2+} to Fe^{3+} conversion was employed for both conditions in this study to determine the antioxidant properties of *Q. borneensis* extracts. All extracts demonstrated an increase in antioxidant capacity when incubated with cells compared to the extracts alone. This could be due to the increase in cellular antioxidant because untreated cells showed almost no activity (0.62 μM $FeSO_4 \cdot 7H_2O$). The presence of high polyphenolic content in plant extracts was known to possess antioxidant properties and can be extracted with polar organic solvents (Ablat et al. 2013; Dai & Mumper 2010). This could be the explanation of higher antioxidant activities shown by *Q. borneensis* chloroform extracts.

It was known that an excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important role in cancer development. At the site of inflammation, respiratory burst caused massive release of ROS such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2). NO is chemically unreactive, but can react with these molecules and produce a powerful oxidant, peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2) and nitrogen trioxide (N_2O_3). Damage to surrounding tissues cause by these radical molecules will trigger more infiltration of inflammatory cells and activate signaling pathway linked to inflammation and cell proliferation such as NF-κB. In turn, NF-κB activation will lead to

upregulation of iNOS expression and therefore producing more NO (Reuter et al. 2010).

In addition, *Q. borneensis* extracts specifically the chloroform extracts demonstrated good antiproliferative activity towards HL-60 cells. The cytotoxic and inhibition of cell proliferation properties of many Simaroubaceae plants have been extensively studied and linked to the presence of specific quassinoids. For example, several quassinoids have been isolated from *Q. amara* leaf extracts, however only the simalikalactone D gave a significant cytotoxic activity (Houel et al. 2009). The bioactive composition was still not determined in our study, but it is expected that quassinoid contributed to the inhibition of HL-60 cell proliferation as that compound was known for its antileukemic effects (Hitotsuyanagi et al. 2006).

Findings of this study suggest that *Q. borneensis* is a valuable medicinal plant with the potential as a source of chemopreventive agent which could be based on its antiinflammatory and antioxidant properties. *Q. borneensis* can also be further studied to search for a new chemotherapeutic candidate due to its potent antiproliferative activity of the chloroform extracts. Recently, Ag Nuddin et al. (2015) had reported the presence of canthin-6-one alkaloid and five quassinoids which were identified as glaucarubolone, chaparrinone, holacanthone, glaucarubinone and ailanthinone in this species. Although quassinoids are known for its antileukemic and antimalarial activities, determination of chemopreventive and/or chemotherapeutic activities of these compounds will then explain the medicinal properties of *Q. borneensis* and the mechanism of action involved at the cellular and molecular levels.

CONCLUSION

Q. borneensis extracts particularly the chloroform extracts possess antiinflammatory, antioxidant and antiproliferative properties. Our scientific findings would provide basic information for its medicinal purposes. However, the characterization of the bioactive constituents which include the quassinoid is still needed to discover its therapeutic potential.

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REFERENCES

Ablat, A., Mohamad, J., Awang, K., Shilpi, J. A. & Arya, A. 2014. Evaluation of antidiabetic and antioxidant properties of *Brucea javanica* seed. *The Scientific World Journal*. http://dx.doi.org/10.1155/2014/786130.

Ag Nuddin, J., Adam, A. & Hamzah, A. S. 2015. Suggestive placement of *Quassia borneensis* Noot. (Simaroubaceae) as indicated by isolation of its quassinoids. *Jurnal Teknologi* 77(2): 13-17.

Benzie, I.F. & Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analatical Biochemistry* 239(1): 70-76.

Bertani, S., Houel, E., Stien, D., Chevolot, L., Jullian, V., Garavito, G., Bourdy, G. & Deharo, E. 2006. Simalikalactone D is responsible for the antimalarial properties of an Amazonian traditional remedy made with *Quassia amara* L. (Simaroubaceae). *Journal of Ethnopharmacology* 108(1): 155-157.

Bhat, R. & Karim, A.A. 2010. Tongkat Ali (*Eurycoma longifolia* Jack): a review on its ethnobotany and pharmacological importance. *Fitoterapia* 81(7): 669-679.

Dai, J. & Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15(10): 7313-7352.

Fabricant, D.S. & Farnsworth, N.R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* 109(Supplement 1): 69-75.

Ghazali, A.R., Ag Nuddin, J., Sabireen, S.N., Wan Razali, W.M. & Ishak, S.A. 2013. Ex vivo antiplasmodial activity of *Quassia borneensis* Noot. (Simaroubaceae) extracts against mice erythrocytes infected by *Plasmodium berghei* NK 65. *Journal of Undergraduate Studies* 2(3): 24-31.

Goh, S.H., Chuah, C.H., Mok, J.S.L. & Soepadmo, E. 1995. *Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases*. Kelana Jaya: Pelanduk Publications.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. & Tannenbaum, S.R. 1982. Analysis of nitrate, nitrite and [15N]nitrate in biological fluids. *Analatical Biochemistry* 126(1): 131-138.

Hasiah, A.H., Ghazali, A.R., Weber, J.F., Velu, S., Thomas, N.F. & Inayat-Hussain, S.H. 2011. Cytotoxic and antioxidant effects of methoxylated stilbene analogues on HepG2 hepatoma and Chang liver cells: implications for structure activity relationship. *Human and Experimental Toxicology* 30(2): 138-144.

Hitotsuyanagi, Y., Kim, I.H., Hasuda, T., Yamauchi, Y. & Takeya, K. 2006. A structure–activity relationship study of brusatol, an antitumor quassinoid. *Tetrahedron* 62(17): 4262-4271.

Hofseth, L.J. 2008. Nitric oxide as a target of complementary and alternative medicines to prevent and treat inflammation and cancer. *Cancer Letters* 268(1): 10-30.

Houel, E., Bertani, S., Bourdy, G., Deharo, E., Jullian, V., Valentin, A., Chevalley, S. & Stien, D. 2009. Quassinoid constituents of *Quassia amara* L. leaf herbal tea. Impact on its antimalarial activity and cytotoxicity. *Journal of Ethnopharmacology* 126(1): 114-118.

Jiao, W.H., Gao, H., Zhao, F., Lin, H.W., Pan, Y.M., Zhou, G.X. & Yao, X.S. 2011. Anti-inflammatory alkaloids from the stems of *Picrasma quassioides* Bennet. *Chemical and Pharmaceutical Bulletin* 59(3): 359-364.

Kim, H.M., Kim, S.J., Kim, H-Y., Ryu, B., Kwak, H., Hur, J., Choi, J-H., Jang, D.S. 2015. Constituents of the Stem barks of *Ailanthus altissima* and their potential to inhibit LPS-induced nitric oxide production. *Bioorganic & Medicinal Chemistry Letters* 25(5):1023-1026.

Kulip, J. & Wong, K.M. 1995. Simaroubaceae. In *Tree Flora of Sabah and Sarawak Volume One*, edited by Soepadmo, E. & Wong, K.M. Kepong: Forest Research Institute Malaysia, Sabah Forestry Department & Sarawak Forestry Department.

Kundu, P. & Laskar, S. 2010. A brief resume on the genus Ailanthus: chemical and pharmacological aspects. *Phytochemistry Reviews* 9(3): 379-412.

Lau, F.Y., Chui, C.H., Gambari, R., Kok, S.H.L., Kan, K.L., Cheng, G.Y.M., Wong, R.S.M., Teo, I.T.N., Cheng, C.H., Wan, T.S.K. & Chan, A.S.C. 2005. Antiproliferative and apoptosis-inducing activity of *Brucea javanica* extract on human carcinoma cells. *International Journal of Molecular Medicine* 16(6): 1157-1162.

Liu, J.H., Zhao, N., Zhang, G.J., Yu, S.S., Wu, L.J., Qu, J. & Tang, W.B. 2012. Bioactive quassinoids from the seeds of *Brucea javanica*. *Journal of Natural Products* 75(4): 683-688.

Mantovani, A., Allavena, P., Sica, A. & Balkwill, F. 2008. Cancer-related inflammation. *Nature* 454: 436-444.

Masini, E., Cianchi, F., Mastroianni, R. & Cuzzocrea, S. 2010. Nitric oxide expression in cancer. In*Nitric Oxide (NO) and Cancer*, edited by Bonavida, B. New York: Springer.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65(1-2): 55-63.

Muhammad, I., Bedir, E., Khan, S.I., Tekwani, B.L., Khan, I.A., Takamatsu, S., Pelletier, J. & Walker, L.A. 2004. A new antimalarial quassinoid from *Simaba orinocensis*. *Journal of Natural Products* 67(5): 772-777.

Reuter, S., Gupta, S.C., Chaturvedi, M.M. & Aggarwal, B.B. 2010. Oxidative stress, inflammation and cancer: how are they linked? *Free Radical Biology & Medicine* 49(11): 1603-1616.

Verma, N., Tripathi, S.K., Sahu, D., Das, H.R. & Das, R.H. 2010. Evaluation of inhibitory activities of plant extracts on production of LPS-stimulated pro-inflammatory mediators in J774 murine macrophages. *Molecular and Cellular Biochemistry* 336(1-2): 127-135.

Wan Razali, W.M., Nuddin, J.A., Ishak, S.A. & Ghazali, A.R. 2015. Stage-dependent effects of *Quassia borneensis* Noot. (Simaroubaceae) chloroform extracts on erythrocytes infected with chloroquine-resistant *Plasmodium berghei* NK65. *Jurnal Teknologi* 77(3): 13-17.

Wolfe, K.L. & Liu, R.H. 2007. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 55(22): 8896-8907.

Xie Q-w. 2001. Inducible nitric oxide synthase expression. In Current protocols in toxicology. John Wiley & Sons Inc. http://dx.doi.org/10.1002/0471140856.tx1009s04.

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