SUGGESTIVE PLACEMENT OF *Quassia borneensis* Noot. (Simaroubaceae) AS INDICATED BY ISOLATION OF ITS QUASSINOIDS

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Graphical abstract

Abstract

Quassinoids have been reported to possess numerous biological activities such as anticancer, antimalarial and phytotoxicity. They are degraded triterpenoids which give bitterness characteristic of Simaroubaceae and classified according to their basic skeletons; C_{18}, C_{19}, C_{20}, C_{22}, C_{25} which correspond to their particular genus *Quassia*, *Brucea*, *Soulamea*, *Eurycoma*, *Picrolemnia* and others. *Quassia borneensis* Noot. (Simaroubaceae) (Qb) have not been properly placed in *Quassia* s.l. thus created confusion. Therefore, a study on Qb for its chemical compounds and biological activity are appropriate for data gathering. There is positive possibility for new quassinoids with potent biological activity to be discovered, in addition to confirmation of placement in Simaroubaceae. The study was accomplished by extracting the secondary metabolites from Qb by soxhlet in methanol. The methanol extract was partitioned to acquire *n*-hexane, chloroform and aqueous extracts. These extracts underwent isolation and purification through chromatographic techniques before subjected to spectroscopic instruments for structural elucidation. Preliminary findings identified five quassinoids and one canthin-6-one as glaucarubolone (**1**), chaparrinone (**2**), holacanthone (**3**), glaucarubinone (**4**), ailanthinone (**5**) and canthin-6-one (**6**) in reference to past reports. The isolation confirms Qb placement in Simaroubaceae, however, not in *Quassia* section as indicated by isolated quassinoids. Hence, further study would confirm its proper section in *Quassia*.

Keywords: Quassinoid, Simaroubaceae, *Quassia*, *Quassia borneensis*

Abstrak

Kuasinoid dilaporkan memiliki pelbagai keaktifan biologi seperti antikanser, antimalaria dan ketoksikanfito. Ia adalah triterpenoid yang terubah, memberikan sifat pahit kepada Simaroubaceae dan dikelaskan mengikut rangka kimia asasnya; C_{18}, C_{19}, C_{20}, C_{22}, C_{25}. Hingga kini, lebih daripada 200 sebatian kuasinoid telah dipisahkan dari Simaroubaceae. Kajian ke atas sifat kimia dan keaktifan biologi *Quassia borneensis* Noot. (Simaroubaceae) (Qb) bakal menghasilkan data terkini bagi genus *Quassia*. Penemuan kuasinoid bakal mengesahkan kedudukannya di dalam Simaroubaceae. Kajian telah dimulakan dengan pengekstrakan metabolit sekunder dari Qb menggunakan soxhlet di dalam metanol. Ekstrak metanol telah diperingkatkan bagi menghasilkan ekstrak *n*-heksana, kloroform dan akueus. Semua ekstrak ini melalui proses pengasingan dan penulenan menggunakan teknik kromatografi sebelum dianalisa secara spektroskopi bagi penentuan struktur. Akhirnya, lima

kuasinoid dan satu kantin-6-on telah diasingkan, ditentukan strukturnya dan disahkan sebagai glaukarubolon (**1**), kaparinon (**2**), holakanton (**3**), glaukarubinon (**4**), ailantinon (**5**) dan kantin-6-on (**6**) merujuk kepada laporan lepas. Pengasingan tersebut mengesahkan kedudukan Qb di dalam Simaroubaceae tetapi tidak di dalam seksyen *Quassia* seperti ditunjukkan oleh kuasinoid yang diasingkan. Oleh itu, pengasingan yang seterusnya bakal memberikan gambaran yang lebih jelas.

Kata kunci: Kuasinoid, Simaroubaceae, *Quassia, Quassia borneensis*

1.0 INTRODUCTION

Simaroubaceae species are known to produce very bitter secondary metabolites; tryptophan-derived alkaloids (β-carbolines, canthinones) and triterpene-derived quassinoids. The medicinal benefits had been reputed to be due of these metabolites [1, 2]. They can be found in primary mixed dipterocarp forest of Malesia.

The tree can grow up to 25 m tall and 25 cm in diameter with low buttresses. The bark is pale yellow to brownish bark and white sapwood. The leaves are shiny, pinnate and spirally arranged, elliptic to obovate-oblong in shape with apex shortly rounded to acuminate and sunken lateral veins on both surfaces. It has puberulous inflorescences where the male flowers form 4-5-merous, contorted or imbricate in bud, glabrous, elliptic to ovate-oblong petals with slightly shorter than petals stamen. There would be 1-5 fruits in each flower, drupaceous, dark purple-red when ripe, a bit ellipsoid with a faint dorsal and ventral ridge, and thin but hard pericarp. Its seed has a thin testa with large, green and plano-convex cotyledon [3].

Endemically found in Sabah, *Q. borneensis* has also been described as *Simaba borneensis* (Noot.) Feuillet in *Bulletin du Jardin Botanique National de Belgique*, volume 53, series 510 in the year 1983 by Christian Patrice Georges-André Feuillet [4]. When Nooteboom (1962) described *Q. borneensis* as a novel species based on Meijer specimen SAN 20499, he noted that it has a close resemblance to the African species, *Quassia sect Odyendyea* described by PIERRE [5]. He mentioned that both species have the filaments sinuously folded in a bud, a condition that does not exist in other *Quassia*.

The Dictionary of Terpenoids has described quassinoids as terpenoidal secondary metabolites found in Simaroubaceae. Biosynthetically, quassinoids are metabolically altered triterpenoids with varying degree of oxygenation and unsaturation, known as decanortriterpenoids as they have lost 10 carbon atoms in their biosynthesis, hence the prefix 'decanor' [6]. Picrasane skeleton has been accepted as the stereoparent and numbered according to *Chemical Abstracts* [7].

Quassinoids are heavily oxygenated lactones with variable number of hydroxyl group, hydroxyl esterified, carbonyl, methoxyl and carbomethoxyl.

They are classified according to their basic skeletons; represented by 5 basic skeletons (Figure 1): i) skeleton of 25 carbon atoms (C_{25}), ii) skeleton of 22 carbon atoms (C_{22}), iii) skeleton of 20 carbon atoms (C_{20}), iv) skeleton of 19 carbon atoms (C_{19}), and v) skeleton of 18 carbon atoms (C_{18}). Each species will denominate its own skeleton [8].
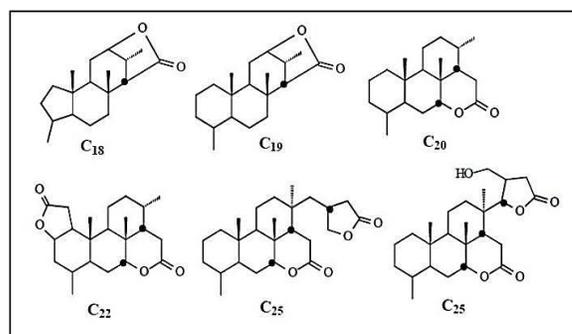

Figure 1 Basic Skeletons of Quassinoids [9]

The chemical and biological studies of *Quassia borneensis* Noot. from Simaroubaceae have never been performed. The presence of quassinoids and canthin-6-ones will confirm the species's position in Simaroubaceae. The chemical structure of isolated compounds may suggest the genus of *Quassia borneensis* Noot. based on their skeleton structure.

2.0 EXPERIMENTAL

2.1 Plant Materials

Samples of the plant were acquired from a fully grown tree found in Tawai Forest Reserve, Telupid, Sabah. The location was noted and recorded using Garmin GPS Map 60CSx. The plant sample identification was performed by Dr Joan T. Pereira, Botanist of FRC for confirmation.

Herbarium specimens were prepared by pressing *Q. borneensis* twig with leaves in between clean sheets of paper with wooden square press and left to dry for a few days. Once dry, the part was sewn on a herbarium card and labelled with collection information. They were prepared with guidance from Encik Postar Miun, a Research Assistant of FRC, and

deposited in Herbarium of KomSaT, FSG, UiTM Sabah and Herbarium of FRC, SFD, Sandakan, Sabah.

Plant's samples were classified to twigs, wood, leave, root and bark before they were cleansed from dirt, cut to pieces and air-dried in a well ventilated room until they have become properly dry. Shredding was done using Camon C150 Chipper and Shredder to prepare the samples for milling. Lastly, they were pulverised to powder with a disc mill, FFM DM-50 Disc & Mill Grinder. The ground samples were sealed in zip-locked bags to maintain dryness and weighed with top balance before stored in a -15 $^{\circ}$C chill room.

2.2　Extraction and Isolation

Powdered form of plant samples (500 g) was soaked in petroleum ether in 2 L conical flasks to remove their fatty substances for 24 hours. The samples were then soxhlet in methanol for 24 hours before concentrated *in vacuo* with a rotary evaporator. Prior to partitioning, the concentrated methanol extracts were suspended in 10% aqueous methanol and partitioned between *n*-hexane and chloroform. The pooled extracts of *n*-hexane and chloroform were concentrated *in vacuo* producing *n*-hexane and chloroform fractions. The aqueous portions were freeze-dried to acquire aqueous fractions. These *n*-hexane, chloroform and aqueous fractions were stored in 35 mL glass, screw capped sample bottles and labelled as *Q. borneensis* soxhlet fractions. All extracts were left to dry in desiccators and upon complete dryness, weighed with an analytical balance.

Isolation and purification works were achieved through HPLC instrumentation and techniques. In the isolation method developed for *Q. borneensis* plant sample extracts' profile, chromatography analysis was performed using Agilent 1200 Quaternary Pump (QP) equipped with thermostated automated liquid sampler (TALS), degasser, thermostated column compartment (TCC), diode-array (DAD) and fluorescence detectors (FLD). The system was installed with Chemstation for LC systems revision B.03.01[317] 2007 as its interfacing software. The method utilised a reversed phase column; Zorbax SB-C_{18} 250 mm x 4.6 mm, with a pore size of 5 μm, with similar characteristic guard column, in a step gradient elution of H_2O:ACN (100-0%-100% (10% increment for 5 min, 50% decrement for 5 min). Samples were injected at 20 μg with the solvent systems introduced into the system at 0.6 mL/min. The DAD was read at the wavelength of 210 and 245 nm, with a bandwidth (BW) of 4 nm at a reference wavelength of 360 nm and BW of 100 nm.

For isolation purpose, 1 g of the plant chloroform soxhlet root extract was fractionated by a preparative chromatographic system comprised of Agilent 1200 Binary Pump (BP), thermostated automated liquid sampler (TALS), bandwidth (BW), interfaced with Chemstation for LC systems revision B.03.01[317] 2007 was utilised. The preparative work

used a reversed phase Zorbax SB-C_{18} 250 x 21.2 mm with a pore size of 7 μm. The pooled fractions were concentrated *in vacuo* before weighing and purifying.

Purification work was performed on Agilent 1200 Quaternary Pump (QP) equipped with thermostated automated liquid sampler (TALS), degasser, thermostated column compartment (TCC), multiple wavelength detector (MWD) and thermostated fraction collector (TFC), interfaced with Chemstation for LC systems revision B.03.01[317] 2007. Pooled sub fractions were analysed for purity on analytical HPLC system and concentrated *in vacuo* before storing in 15 mL glass, screw capped sample vials.

2.3　Spectroscopy Analysis

Mass analysis was performed at Pharmacogenomics Centre (PROMISE), UiTM Kampus Puncak Alam for measurement of accurate mass using UPLC-MS-Time of Flight Detector (UPLC-MS-TOF). The instrument was an Agilent 1200 Binary Pump SL, equipped with degasser, high performance, thermostated automated liquid sampler (HiP-ALS SL+), thermostated column compartment (TCC SL,), diode array detector (DAD SL) and time-of-flight mass detector Agilent 6224 TOF LC/MS G6224A. The samples were injected into a Zorbax Eclipse XDB-C_{18} Rapid Resolution High Throughput (RRHT), 4.6 x 50 mm, 1.8 μm, 600 bar, which was kept warm in 40°C. 2 μL of compounds were injected. The mobile phase was introduced into the column at 0.3 mL/min, isocratic at 1:1 (H_2O:ACN), with 0.01% formic acid as pH modifier, for 10 minutes without post time. The MSD was programmed in positive mode Dual Electrospray ionisation (ESI); the drying nitrogen gas temperature was set at 325°C, flowing at 8 L/min with the nebuliser's pressure at 40 psig, reading TOF spectra from 100 to 1000 mass units which was presented as total ion current (TIC) chromatogram. MS data was processed using Mass Hunter Qualitative version B.05.

In NMR analysis, preliminary samples were sent to NMR Laboratory at Atta-ur-Rahman Institute for Natural products Discovery (RiND), UiTM Kampus Puncak Alam, for analysis using 500 MHz NMR of Bruker Ultrashield 500 Plus. NMR data from Bruker NMR spectrometer was processed with Topspin ©2009 Bruker Biospin. A second NMR spectrometer was employed for final analysis, JEOL EXS600 in Institut Biologi Tropika dan Pemuliharaan, Universiti Malaysia Sabah. NMR data from JEOL NMR spectrometer was processed with DELTA version 4.3.6 specifically for JEOL NMR machine. About 3 mg of compound was dissolved in deuterated methanol (MeOD) except for experiments performed at RiND, which was dissolved in $CDCl_3$. Measurements were achieved in NMR glass tubes for 500 and 600 MHz measurement.

3.0 RESULTS AND DISCUSSION

The presence of isolated quassinoids comfirmed the placement of *Q. borneensis* in Simaroubaceae. In addition, the type of quassinoid skeletal obtained in its isolation may throw some light on the existing confusion. This is in agreement as chemotaxonomic information has been accepted to be more indicative of familial and subfamilial circumscriptions [10].

Quassia is the first genus of Simaroubaceae to be published. It belongs to the tribe Simaroubeae and is synonym to *Samadera*, *Simaba*, *Hannoa* and *Odyendea*. A *Quassia* tree should be small, sometimes shrubby, with simple leaves, often few to numerous in numbers. It has bisexual flowers, in axillary or terminal, peduncled false umbels or racemes with 3-5 petals which are hairy on the back, a bit puberulous with short stigma. The fruits are laterally compressed, with a narrow, unilateral, sharp-edged thinner part in the apical half [11].

Eventhough Cronquist (1944) insists that the genera of Simaroubaceae is sufficiently defined thus despite the close relationship shared by *Simarouba*, *Simaba* and *Quassia*, they are still distinguishable [12]. As the family is morphologically diverse, it is roughly described as dioecious plants with alternate compound leaves, five-parted flowers and drupaceous fruits [13]. Nonetheless, for Simaroubaceae such as *Q. borneensis*, having simple leaves, such description is inadequate.

After examining *Q. borneensis*, Nooteboom had concluded that the genus should be expanded by including *Quassia*, *Samadera*, *Simarouba*, *Simaba*, *Hannoa*, *Odyendyea* and *Pierreodendron*. He found that *Q. borneensis* shares common traits with American *Simaba* and, African *Hannoa* and *Odyendea*. Thus, he agreed with the suggestion made by PIERRE in 1896. Therefore, he recircumscribed *Quassia* s.l. to four sections; *Quassia*, *Samadera*, *Simarouba* and *Simaba* [5]. In his PhD thesis, Clayton (2008) has phylogenetically determined that the genus *Quassia* has two species only; *Q. amara*, originally from South America; and *Q. africana*, found in tropical west of Africa. He opined that *Q. borneensis* is superficially similar to *Q. gabonensis* albeit having simple leaves, hence an ambiguous placement. He added that other Old World *Quassia* species, which are simple-leaved, should be placed under the genus *Samadera*. Furthermore, *Simaba, Simarouba, Pierreodendron, Hannoa, Gymnostemon, Perriera, Eurycoma* and *Odyendea gabonensis* in *Quassia* should be recognized distinctly as they are unique in terms of the chemistry of quassinoid, anatomy of pericarp and diagnostic of morphological characters [14].
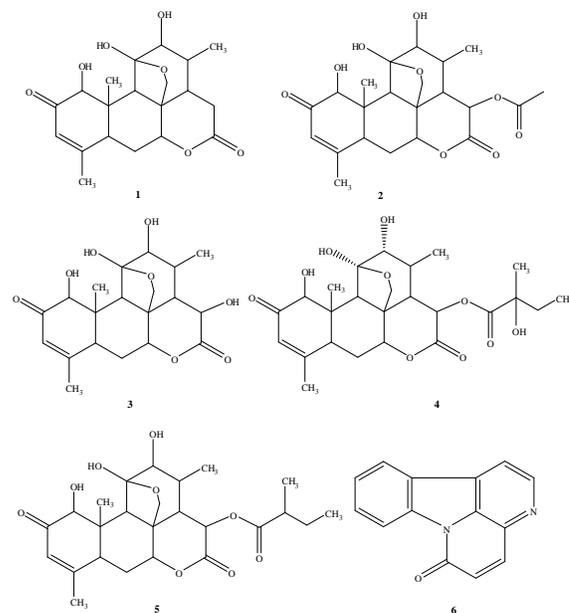
This argument is substantiated by Simao *et al*. (1991), who found that by correlating chemical data with species of Simaroubaceae and their geographical distribution, all the tribes of Simaroubaceae; Simaroubeae, Picrasmeae and Soulameae synthesise quassinoids in a skeletal form of Q2, with addition of Q-D1 for American and West African species, while for East African and Asian species in form of Q4-Q9 (Figure 2) [15]. In addition, M. F. D. G. F. Da Silva & Gottlieb (1987) have drawn up a scheme of possible biosynthetic relationship of the quassinoids types which is basically made up of two main chemical structure skeletons, Q-D1 and Q-D2 [16]. They summarised that, systematic chemical differences exists among the tribes of Simaroubaceae. They came up with such conclusion after determining the skeletal specialization (S) and oxidation state (O) of quassinoids. They have found that EA_S/EA_O plot for genera of Simaroubaceae shown positive correlation. They have argued that the genus *Quassia* has low mean S and O values besides having oxide bridge between C-8 and C-13, stood chemically different apart from *Simarouba*, *Odyendyea* and *Hannoa*. The three genera are closely related chemically since they share the characteristic feature, the oxide bridge linking C-8 and C-11. Thus, *Quassia* shows dissimilarity to *Simaba*, *Simarouba* and *Samadera* despite being considered sections of *Quassia* genus by Nooteboom (1962). In their tabulation, chaparrinone (**1**) has been isolated from the genera *Hannoa*, *Simaba* and *Soulamea*, glaucarubolone (**3**) from *Castela*, *Hannoa*, *Simarouba* and *Soulamea*, glaucarubinone (**4**) from *Ailanthus*, *Odyendyea*, *Perriera*, *Pierreodendron*, and *Simarouba*, and ailanthinone (**5**) from *Ailanthus*, *Hannoa*, *Odyendyea*, *Pierreodendron* and *Simarouba*.
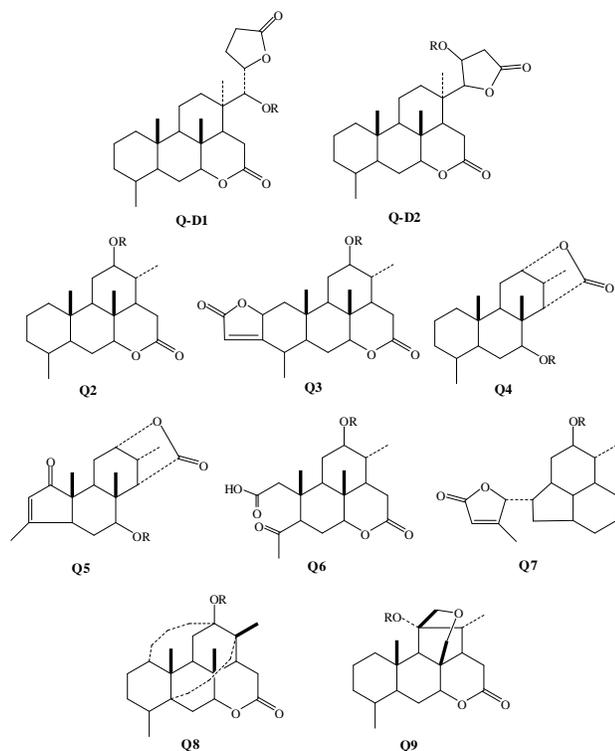
Figure 2 Skeletal form of quassinoids

Based on the above mentioned debate, the isolation of quassinoids metabolites in this study would imply that *Q. borneensis*, even though rightfully placed in the genus *Quassia* s.l., should not be in *Quassia* section. A more detailed study either phytochemical or molecular would clarify the positioning of the species.

4.0 CONCLUSION

The isolation of these secondary metabolites have confirmed that Qb is a Simaroubaceae, however, the chemical structure of these metabolites indicate that Qb does not belong in *Quassia* section of *Quassia* genus as described as Nooteboom. This observation was also made by Clayton. As part of his PhD study, Clayton had examined a single specimen of *Q. borneensis*, obtained from Royal Botanic Garden, Kew, London, United Kingdom. He was unable to obtain its molecular data therefore, unable to determine accurately its position in the family. However, he deduced that based on the description, *Q. borneensis* may be part of *Simaba* since it is similar to *Quassia gabonensis* from Africa, indicating a more detailed study is appropriate.

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