

Research Bulletin

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Some *Coelogyne* spp. of Sarawak
(photograph courtesy of Qammil Muzzammil Abdullah)

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Dean's Message

Prof. Dr. Shabdin Mohd Long

Universiti Malaysia Sarawak has been ranked among the top 200 Asian University by the QS Asian University Rankings 2011 and Faculty of Resource Science and Technology (FRST) was ranked top 115 by QS Asian University Rankings 2011. Faculty members should be proud on our standing and this was achieved due to high commitment and enthusiasm of all staffs. I am sure with strong commitment and hard work we can achieve even better ranking.

In line with the university's vision to become a research university by 2015, the faculty greatly supports any initiative to bring advancement in research and development (R&D) and teaching. Various strategies and plans have been mapped out in tandem with the university's endeavour to spur and harness competency in R&D, science and technology, and capacity to innovate.

Being one of the twelve mega biodiversity countries in the world, we place a lot of emphasis on sustainable utilization, prudent management and conservation of our rich natural heritage. In order to utilize and manage our biodiversity sources, FRST is taking necessary action to establish another Centre of Excellence in addition to Centre of Excellence for Sago Research (CoESAR). CoESAR research focus on various aspect of sago especially agronomy, molecular genetics, starch technology and utilization. It is no doubt that the establishment of new Centre of Excellence will promote research activity and collaboration with other established centre and also focused research which will have significant impact on research, development and commercialization.

My sincere hope that all academic staffs will show high commitment in order to achieve excellent performance in teaching, research, publication, consultancy and public services.

Please feel free to direct your enquiry to me at e-mail: lshabdin@frst.unimas.my or to the editorial members for further information.

Happy reading

Secondary metabolites of *Goniothalamus* spp. in Sarawak

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Phytochemical studies on some of the *Goniothalamus* spp. found in Sarawak resulted in the isolation and characterization of various cytotoxic styryl-lactones and their derivatives, alkaloids, flavonoids and also essential oils with interesting biological activities. Some of the more important and common styryl-lactones derivatives isolated includes dehydrogoniothalamine, 5-acetoxystyrylgoniothalamine, goniothalamine, 5-hydroxystyrylgoniothalamine, goniotriol, and goniodiol. These compounds have been isolated from *G. malayanus*, *G. tapisoides*, *G. velutinus*, *G. giganteus* and *G. uvarioides*. All the isolated styryl-lactones and their derivatives showed interesting biological activity especially cytotoxicity on various human tumour cell lines. The most active compounds especially goniotriol showed strong activity with ED₅₀ values less than 20 µg/mL towards P388, WEH1164, THP-1, MOLT4, HeLa, MCF7 and A-549. *G. tapisoides* and *G. uvarioides* were also rich in alkaloid and some of the alkaloid isolated from this species includes goniothalamine, aristolactam AII, aristolactam AIII, aristolactam BII and velutinam. Aristolactam AIII and velutinam showed strong cytotoxicity with ED₅₀ values less than 20 µg/mL towards P388, WEH1164, THP-1, MCF7 and A-549 cells. Besides that, some flavonoids especially pinocembrin and 5,7,4'-trihydroxyflavanone were also isolated from some of the species studied.

Phytochemical studies on the flowers of *G. uvarioides* revealed the presence of various interesting compounds and not many studies have been performed on the flowers of *Goniothalamus* spp. due to their limited availability. Ten compounds identified as cinnamic acid; dihydrochrysin; β-sitosterol; six styryllactones identified as (+)-3-acetyltholactone, goniotriol, (+)-altholactone, (+)-goniofufurone, 9-deoxygonioppyrone, howiin A; and an aporphine alkaloid identified as (-)-nordicentrine were isolated and characterized.

The isolated compounds were evaluated for antiplasmodial, antimycobacterial and anticancer cell lines tests. Acetyltholactone, goniotriol, (+)-altholactone and (-)-nordicentrine exhibited antiplasmodial activity against *Plasmodium falciparum* (IC₅₀ 2.4, 5.9, 3.2 and 0.2 µg/mL, respectively), while goniotriol, (+)-altholactone, howiin A and (-)-nordicentrine showed antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 100, 6.25, 6.25 and 12.5 µg/mL, respectively). In addition, acetyltholactone, goniotriol, (+)-altholactone, (+)-goniofufurone, 9-deoxygonioppyrone, howiin A; and (-)-nordicentrine showed cytotoxicity against cancer cells, KB, BC1, NCI-H187, and MCF-7 with IC₅₀ ranging from 0.3 to 18.9 µg/mL.

For the essential oil studies, twenty-eight compounds were identified in the leaf oil of *G. tapisoides*, constituting 99.5% of the oil. The most abundant component was 1,8-cineol

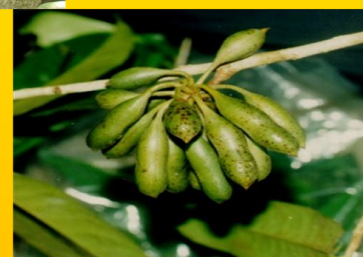
(79%). Other major compounds present in the oil were α-pinene (9.6%), α-terpineol (4.4%), terpinen-4-ol (2.3%) and linalool (1.0%). As the leaf oil, the bark oil of *G. tapisoides* was also made up almost entirely of monoterpenes and their oxygenated derivatives (99.4%).

The bark and leaf oils of *G. tapisoides* were found to possess some compositional similarity but quantitative differences in the concentration of each component. Twenty-four compounds had been identified in the bark oil and the most abundant component was 1,8-cineol (47.9%). Other major constituents of the bark oil were terpinen-4-ol (22.5%), p-menthene (6.9%), γ-terpinene (6.6%), α-pinene (6.6%), α-terpineol (2.8%) and linalool (2.3%).

A comparison between the root and the bark oils of *G. tapisoides* showed that they were qualitatively similar. Essential oils of other species were also analyzed and showed the presence of almost similar major constituent but with different percentage.



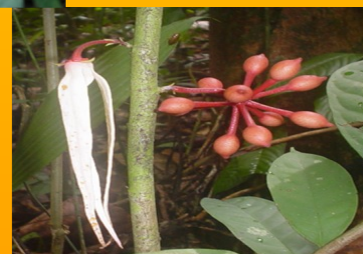
Goniothalamus uvarioides



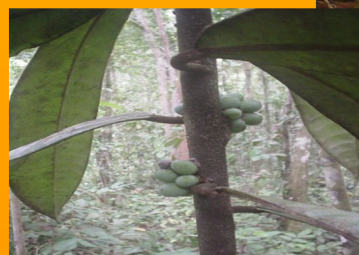
Goniothalamus malayanus



Goniothalamus giganteus



Goniothalamus tapisoides



Goniothalamus velutinus

Micropropagation of *Aquilaria malaccensis* Lank. and *Aquilaria microcarpa* Baill.

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Aquilaria species belongs to the family Thymelaeaceae, is of economic important source of non timber forest product, gaharu or agarwood (Chakrabarty, 1994; Soehartono and Newton, 2000). There are seven *Aquilaria* species have been found in Malaysia. The most popular species for production of gaharu is *A. malaccensis* (Chang *et al.*, 2002). *A. malaccensis* is also found in other countries, such as in Bangladesh, Bhutan, India, Indonesia, Iran, Myanmar, Philippines, Singapore and Thailand (Oldfield *et al.*, 1998). *A. microcarpa*, which is also can produce gaharu, the species distribution however is more restricted in Malaysia and Indonesia. In Malaysia, *Aquilaria* spp. have various vernacular names, however in Malay they are known as karas, tabak, candan, kepong or depu (Lim and Noorainie, 2010).

Both of these *Aquilaria* species are the sources of fragrant wood that has been traded since biblical times for uses in religious functions and for medicinal and aromatic preparations (Zich and Compton, 2001; Lim and Noorainie, 2010). The most common forms of traded gaharu are chips, flakes, oil and powder waste after extraction (Yaacob, 1999). Chang *et al.* (2002) reported that prices of gaharu chips ranged from 60 cent per kg for the low and mixed grades to more than RM 2000.00 per kg for the high grade. However, in 2010 a random survey in the retail local markets we found prices for the top-quality gaharu could reach above MYR 18,000.00 per kg.

Gaharu is not formed in each of the *Aquilaria* spp. tree. The formation of gaharu is said associated with the plant defense mechanism due to pathological effect. Currently, it is difficult to get good quality gaharu from *Aquilaria* spp. trees that are growing in natural forest. However, its price offered in a market has stimulated illegal and over harvesting, and as a result, *Aquilaria* trees are often cut down indiscriminately. Populations of this tree have declined and may lead to possible extinction in the near future if no effective conservation effort is done.

At present, all of the *Aquilaria* species have been included in The World List of Threatened Tree of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) Appendix II (Lim and Noorainie, 2010). Initiatives to produce gaharu in planted *Aquilaria* trees have been done especially in Indonesia, India, Cambodia, Thailand and Vietnam. Cultivation of *Aquilaria* species in Malaysia received attention only in the recent years. In addition to conserving, the cultivation of the tree species is to ensuring sustainable supply of the agarwood. Thus, methods for propagation need to be developed.

In the present work, micropropagation system via tissue culture technique such as organogenesis was carried out to

develop shoot regeneration and multiplication in *A. malaccensis* and *A. microcarpa* explants.

The study started with an attempt to establish axenic (contamination free) culture. The established axenic cultures were used for subsequent experiments, induction of shoot regeneration and multiplication. Healthy seedlings selected from cultivated *A. malaccensis* and *A. microcarpa* in the UNIMAS Green House and at UNIMAS Arboretum were used as stock plants for explants preparation. Two types of explants i.e. shoot tip and lateral bud were used. The plant parts were sectioned into convenient length of about 0.5-2 cm and washed with tap water. Surface sterilization was carried out by immersing the explants in 10 and 15% (each for 10 and 15 minutes) Clorox containing one or two drops Tween 20 with gentle agitation. The explant materials were rinsed three to five times, 5 minutes each time, with sterilized distilled water.

After surface sterilization, all the washed explants materials were submerged in 0.3% Benomyl for one hour for further surface sterilization. All operations were conducted in a laminar-air-flow hood to maintain aseptic condition for the explants. For shoot regeneration and multiplication, explants of *A. malaccensis* and *A. microcarpa* were cultured on Murashige and Skoog (MS) medium with different concentrations of benzylaminopurine (BAP) and Thidiazuron (TDZ). For root initiation, the shoots were cultured on ½ MS medium supplemented with different concentration of IBA.

In this study, surface sterilization with 10% of Clorox for 15 minutes was considered as the best method to obtain axenic explants for both of the *Aquilaria* species. The survival rate and clean tissues of *A. malaccensis* established was 68.8% for shoot tip and 56.3% for lateral bud (Table 1). Higher percentages of axenic explants however, were obtained from *A. microcarpa* compared to of *A. malaccensis*, where 81.3% survival rate for shoot tips and 75.0% for lateral bud of *A. microcarpa* were recorded (Table 2).

Table 1: Number and percentage of axenic explants of *A. malaccensis* (Number of explants inoculated = 16)

Surface sterilization regime	Shoot tips		Lateral bud	
	No. of explants	Percentage (%)	No. of explants	Percentage (%)
10% (v/v) Clorox, 10 min	8	50.0	6	37.5
10% (v/v) Clorox, 15 min	11	68.8	9	56.3
15% (v/v) Clorox, 10 min	5	31.3	6	37.5
15% (v/v) Clorox, 15 min	3	18.8	5	31.3

Table 2: Number and percentage of axenic explants of *A. microcarpa* (Number of explants inoculated = 16)

Surface sterilization regime	Shoot tips		Lateral bud	
	No. of explants	Percentage (%)	No. of explants	Percentage (%)
10% (v/v) Clorox, 10 min	6	37.5	4	25.0
10% (v/v) Clorox, 15 min	13	81.3	12	75.0
15% (v/v) Clorox, 10 min	5	31.3	4	25.0
15% (v/v) Clorox, 15 min	5	31.8	3	18.8

Concentration of BAP and TDZ has effect on the regeneration of shoots for both *Aquilaria* species (**Figure 1-4**) Of different concentrations of cytokinin (BAP and TDZ) used for shoot development, the highest number of shoot tip explants of *A. malaccensis* forming the new shoots was on media with BAP at 0.5mg/L and TDZ at 0.25 mg/L, respectively (**Table 3**). The highest percentage of shoot tip explants developed multiple shoots in medium incorporated with BAP was 81.3%, while with TDZ was 93.8%. Although the highest number lateral bud explants of this species multiple shoots was also obtained from 0.25 mg/L TDZ (87.5%), **Table 4** shows that on medium with BAP, the explants forming shoots was the highest (93.8%) at concentration 1.0 mg/L.

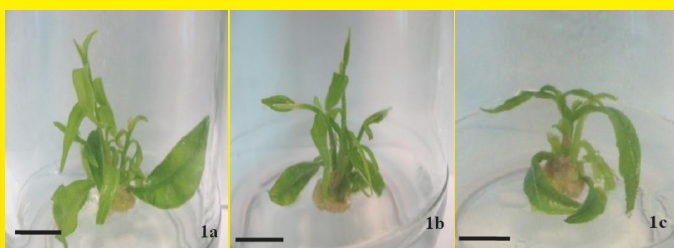


Figure 1: Multiple shoot formation from shoot tip explants of *A. malaccensis* in MS medium supplemented with: (a) 0.5 mg/L BAP, (b) 1.0 mg/L BAP and (c) 0.25 mg/L TDZ

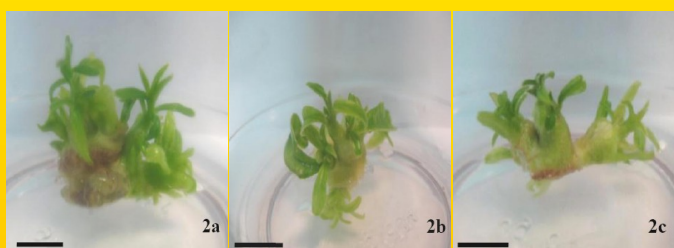


Figure 2: Multiple shoot formation from lateral bud explants of *A. malaccensis* in MS medium supplemented with: (a) 0.5 mg/L BAP, (b) 1.0 mg/L BAP and (c) 0.25 mg/L TDZ

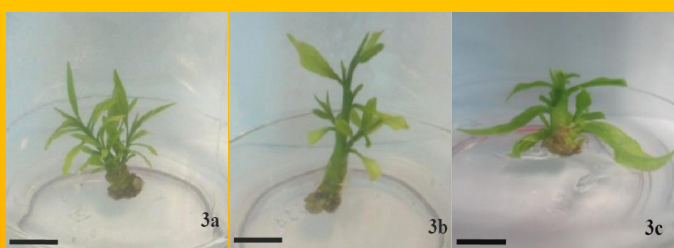


Figure 3: Multiple shoot formation from shoot tip explants of *A. microcarpa* in MS medium supplemented with: (a) 0.5 mg/L BAP, (b) 1.0 mg/L BAP and (c) 0.25 mg/L TDZ

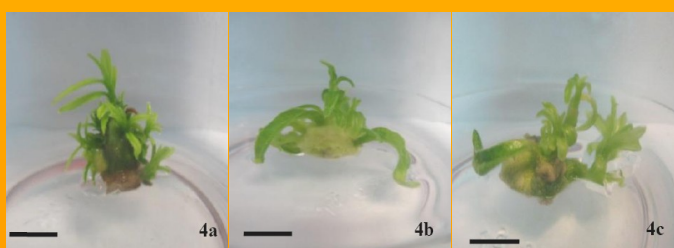


Figure 4: Multiple shoot formation from lateral bud explants of *A. microcarpa* in MS medium supplemented with: (a) 0.5 mg/L BAP, (b) 0.25 mg/L TDZ and (c) 0.5 mg/L TDZ

Table 3: Effect of different concentration of cytokinin on shoots development from shoot tip explants of *A. malaccensis*

Cytokinin (mg/L)	No. of explants inoculated	No. of explants developed shoots	No. of shoots	Frequency of shoots (%)
BAP 0.5	16	13	18	81.3
BAP 1.0	16	10	10	62.5
BAP 1.5	16	6	7	37.5
TDZ 0.25	16	15	14	93.8
TDZ 0.5	16	9	5	56.3
TDZ 1.0	16	5	5	31.3

Table 4: Effect of different concentrations of cytokinin on shoots development from lateral bud explants of *A. malaccensis*

Cytokinin (mg/L)	No. of explants inoculated	No. of explants developed shoots	No. of shoots	Frequency of shoots (%)
BAP 0.5	16	12	8	75.0
BAP 1.0	16	15	10	93.8
BAP 1.5	16	8	5	50.0
TDZ 0.25	16	14	12	87.5
TDZ 0.5	16	9	5	56.3
TDZ 1.0	16	7	6	43.8

As for *A. malaccensis*, the best concentrations of the cytokinins to be added in media to establish shoot multiplication of *A. microcarpa* from shoot tips was 0.5 mg/L for BAP or 0.25 mg/L for TDZ. The highest percentage number of the shoot tip forming the new shoots on BAP and TDZ was 81.3% and 75.0%, respectively (**Table 5**). Similarly for the lateral bud explants, the formation of new shoots was found more on media with 0.5 mg/L BAP or with 0.25 mg/L TDZ, compared to on the media containing higher concentrations of the cytokinins. The highest percentage number of lateral bud explants developed new shoots media with BAP or TDZ was 75.0% and 87.5%, respectively (**Table 6**). MS medium supplemented with higher concentration of TDZ was also found could induce calli surround of lateral bud explants (**Figure 4b**). However, MS medium supplemented with higher concentration of BAP could induce dumpy and twisty shoot on explants.

Table 5: Effect of different concentration of cytokinin on shoot explants of *A. microcarpa*

Cytokinin (mg/L)	No. of explants inoculated	No. of explants developed shoots	No. of shoots	Frequency of shoots (%)
BAP 0.5	16	13	11.0	81.3
BAP 1.0	16	7	5.0	43.8
BAP 1.5	16	5	5.0	31.3
TDZ 0.25	16	12	13.0	75.0
TDZ 0.5	16	10	8.0	62.5
TDZ 1.0	16	7	5.0	43.8

Table 6: Effect of different concentrations of cytokinins on shoot multiplication from lateral bud explants of *A. microcarpa*

Cytokinin (mg/L)	No. of explant inoculated	No. of explants developed shoots	No. of shoots	Frequency of shoots (%)
BAP 0.5	16	12	10.0	75.0
BAP 1.0	16	9	7.0	56.3
BAP 1.5	16	5	6.0	31.3
TDZ 0.25	16	14	16.0	87.5
TDZ 0.5	16	10	13.0	62.5
TDZ 1.0	16	6	8.0	37.5

The presence of BAP and TDZ to stimulate the development of new shoot formation in shoot tips and lateral buds is well known happened in plant. Of the two cytokinins that commonly being used, BAP was reported to be more effective for shoot multiplication in many plants. These include to regenerating shoot in *Trifolium pretense*

(Campbell and Tomes, 1984), *Dipterocarpus intricatus* (Linington and Kew, 1989), *Morus alba* (Sharma and Thorpe, 1990) and olive (Ali *et al.*, 2009). It has also been reported that high concentration of BAP and TDZ could cause stunting and or callusing of shoots (Scott *et al.*, 1995).

In this study, shoot tips and lateral buds explants, both, were found more responsive in shoot proliferation when appropriately balanced plant growth regulators added into the media. In addition, fast elongation of shoots regenerated from shoot tip and lateral bud explants was also seen.

When the regenerated shoots were transferred onto ½ MS medium supplemented with different concentration of IBA for rooting, they rooted within a week (Figure 5). Result in Table 7 and Table 8 shows that IBA at 1.0 mg/L producing higher percentage of rooted shoot compared to the lower concentration of IBA. After 48 days on the media, 70% shoot of *A. malaccensis* and 50% shoot of *A. microcarpa* were rooting. For further laboratory study, rooted plantlets will be acclimatized in tray filled with growing medium before transferred in nursery.

Table 7: The effect of different concentration of IBA on rooting of shoot of *A. malaccensis* after 48 days on the media

IBA (mg/L)	No. of shoot inoculated	No. of shoot rooted	No. of roots	Frequency of rooting (%)
0.5	10	4	8	40.0
1.0	10	7	11	70.0

Table 8: The effect of different concentrations of IBA on rooting of shoot of *A. microcarpa* after 48 days on the media

IBA (mg/L)	No. of shoot inoculated	No. of shoot rooted	No. of roots	Frequency of rooting (%)
0.5	10	3	6	30.0
1.0	10	5	10	50.0

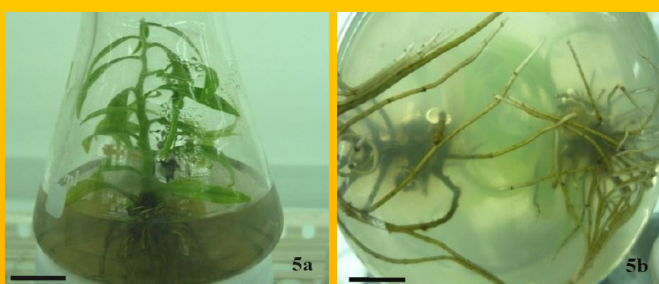


Figure 5: (a) initiation of rooting from shoot of *A. malaccensis*
(b) Root of *A. malaccensis*

As conclusion, surface sterilized with 10% Clorox for 15 minutes was effective to get higher contamination-free explants. BAP at 0.5 mg/L and TDZ at 0.25 mg/L were effective in the induction of higher shoot proliferation in shoot tip and lateral bud explants. Half MS medium with 1.0 mg/L concentration of IBA was effective in the rooting initiation for shoot of *A. malaccensis* and *A. microcarpa*. Using shoot segments as explants, direct plantlet regeneration of *Aquilaria* species can be achieved via organogenesis in short term period and the regenerated plantlets can be used for further propagation. Thus, this study provided a very useful method for propagation of this plant.

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Diversity and phylogenetics study of *Hipposideros*

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Introduction

Tropical rain forests are perfect example of high richness for many taxonomic groups. Bats play an important role in shaping both pattern and process of this diversity (Wilson, 1992). Fruit bats for instance serve by pollinating many plants and dispersing the seed of many others. On the other hand, insect bats play an important role as major consumers of night-flying insects (Jones *et al.*, 2002). In this study, phylogenetic relationship was inferred from mtDNA *cyt b* gene, which is widely used in reconstructing phylogenetic relationships among mammalian species (Baker *et al.*, 1994; Stadelmann *et al.*, 2007). The aim of this study was to identify the genetic relationship among species within genus of *Hipposideros* by using mtDNA *cyt b* gene.

Methodology

A total of 18 study sites were selected and surveyed from June 2008 until April 2010; seven sampling sites were in Peninsular Malaysia while the other 11 sampling sites were in Sarawak. Four-banks harp traps and 10 mist nets were used for trapping bats for at least three consecutive nights which was observed to be the optimum effort (Abdullah, 2003).

Bats identification was done by using Payne *et al.* (2005) and Francis (2008). External morphology measurements were taken using Mitutoyo digital calliper and weighted using Pasola spring balance. Relative abundance of species was calculated by dividing the total number of individual per species with total number of individuals caught. Species similarity indexes were calculated using MultiVariate Statistical Package version 3.2.

DNA extraction using muscle tissues preserved in the ethanol followed the standard cetyltrimethylammonium bromide procedure as described by Grewe *et al.* (1993). PCR amplifications were performed using Biometra Tpersonal Thermoblock (Germany). Primers for full length *cyt b* gene following Irwin *et al.* (1991) as shown in **Table 1**. Phylogenetic trees were constructed using NJ, MP, ML (PAUP 4.0) and Bayesian (Mr Bayes) methods.

Table 1: Primers used in this study and their sequences

Gene	Primer	Sequence	Direction
Cyt <i>b</i>	L14724	5'-cgaagcttgatataaaaaccatcgttg-3'	Forward
	H15915	5'-aactgcagtcacatcctcggttacaagac-3'	Reverse

Results and Discussion

Diversity of bats

A total of 1,278 individuals comprising of 47 species from seven families were recorded in 18 sampling sites (**Figure 1**). Out of 47 species caught, 11 species were captured only in Sarawak while 13 species were recorded only in Peninsular Malaysia while the remaining 23 species were trapped at both regions. Eleven species were from suborder megachiroptera while the other 36 species were microchiropterans. Ta-Ann Naman Plantation shows highest number of individuals caught with 199 individuals and the most abundance species are *C. brachyotis* with 192 individuals (96.5%). The smallest number of individuals trapped recorded at Sungai Dusun FR with only 11 individuals (0.9%). Maximum number of species captured was at Nanga Merit 1 with 17 species representing 36.2% while least number of species recorded was at Bukit Aup FR with only two species representing 4.3%.

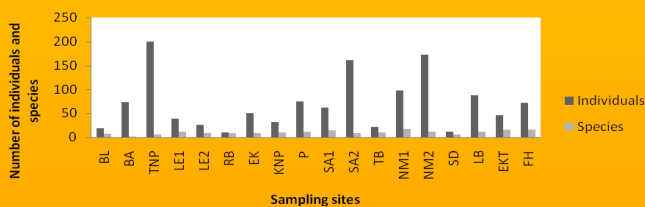


Figure 1: Number of species and individuals of bats at 18 sampling site

Based on Modified Morisita’s similarity coefficients index, the lowest indices are between Sungai Dusun Fr versus (vs) Bukit Lima FR (0.000), Sungai Dusun FR vs Bukit Aup FR (0.000) and Sungai Dusun FR vs Ta-Ann Naman Plantation (0.000). All these three sites did not share any species. The highest indices are between Ta-Ann Naman Plantation vs Bukit Aup FR (0.982) with two shared species followed by Bukit Aup FR vs Bukit Lima FR (0.858) with one shared

species and Lanjak Entimau WS 1 vs Lanjak Entimau WS 2 (0.858) with six shared species. **Figure 2** shows a dendrogram of species similarity for 18 sampling sites. The cluster analysis shows how each sampling sites were related.

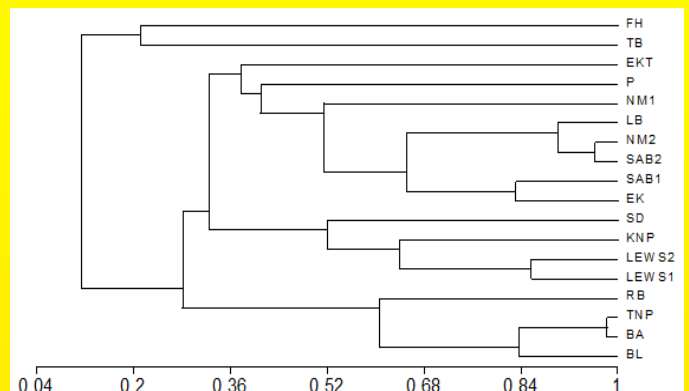


Figure 2: Dendrogram of species similarity for 18 sampling sites (Morisita-Horn)

Phylogenetic analyses

Grouping placements for NJ, MP, ML and Bayesian methods are divided into four major groups. Group 1 consists of four species namely; *H. ridleyi* (subgroup 1a), *H. ater* (subgroup 1b), *H. bicolor* (subgroup 1c) and *H. cineraceus* (subgroup 1d). Group 2 comprises of *H. cervinus* (subgroup 2a) and *H. galeritus* (subgroup 2b). Group 3 consist of single species which is *H. diadema*. In Group 4, *H. larvatus* (subgroup 4b) and *H. armiger* (subgroup 4a) forms basal clade to the other species of *Hipposideros*. In general, phylogenetic relationship of genus *Hipposideros* inferred from *cyt b* is successfully assessed (**Figure 3**). The interspecies and intraspecies relationship of this genus was supported with high bootstrap value and the cluster placement was constant in all tree analyses.

Conclusion

This study provides new information and also updates on the recent distribution of bats in certain sites in Malaysia. This information is very important in order to know the current status of bats and towards conservation of bats in Malaysia by protecting suitable areas for roosting and foraging sites of bats. A numbers of species that listed as near threatened and vulnerable in IUCN have also been recorded. This study was also able to discover useful information on the phylogenetics of *Hipposideros* in Malaysia using mtDNA *cyt b*. The relationships among *Hipposideros* using mtDNA *cyt b* in this study were fully resolved.

Acknowledgments

Our appreciation goes to Faculty of Resource Science and Technology, UNIMAS for the authorization granted to join the inventory. Thanks to the Department of Wildlife and National Parks for the invitation and hospitality during field work; Sarawak Forest Department and Sarawak Forest Corporation for research permission to examine and collect voucher samples. The field work in Sarawak and molecular laboratory analysis were supported by Eco-Zoonosis Research Grant from UNIMAS.

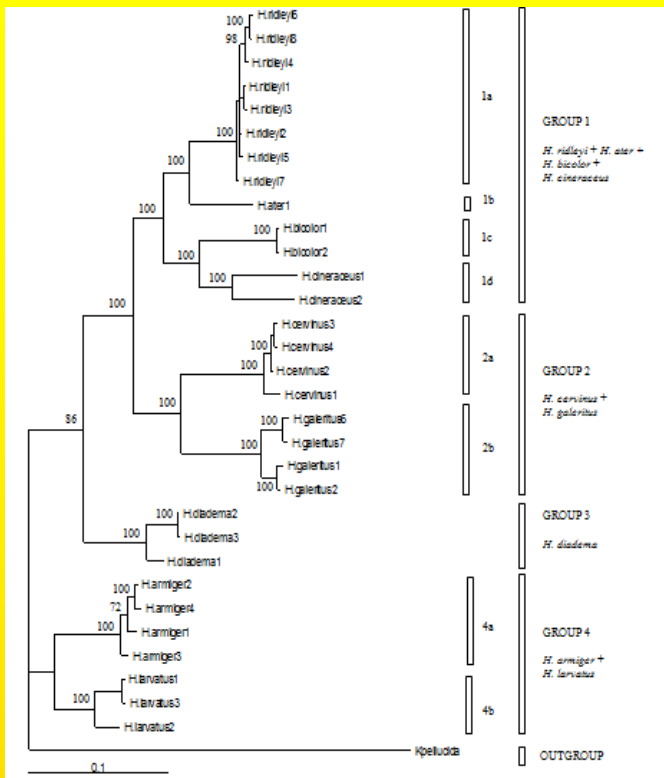


Figure 3: A Bayesian inference with 50% majority rule consensus tree of *Hipposideros* under study based on 973 bp cytochrome *b* gene sequences. Values of Bayesian posterior probabilities (BPP) are shown on the branch nodes (only >50% bootstrap values are shown)

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Description of the species:

Hipposideros larvatus Horsfield 1823
Intermediate roundleaf bat

The upperpart of the body is brown or orange in colour while the underparts are slightly paler. Study by Thabah *et al.* (2006) on genetic divergence and echolocation call frequency in cryptic species of *H. larvatus* from the Indo-Malayan region successfully identified of two types of *H. larvatus* in the region, namely, 85 kHz and 98 kHz and having a 12 to 13% sequence divergence based on 516 base pairs (bp) of mito-

Description of the species:

Hipposideros bicolor Temminck, 1834
Bicoloured roundleaf bat

The upperparts of the body are buffy brown while the underparts are slightly paler. According to a study on *H. bicolor* by Kingston *et al.* (2001) there are two types of *H. bicolor* in Peninsular Malaysia based on the ultrasonic frequencies which are 131 kHz and 142 kHz types and having 7% genetic distance on a mtDNA *cyt b* analysis.



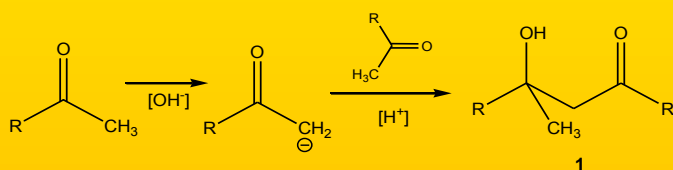
A convenient way for the etherification of *p*-hydroxyacetophenone

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Etherification is one of the important organic reactions especially in the drugs and liquid crystal compounds syntheses. This reaction was firstly reported by Williamson in 1850 for the etherification of aliphatic alcohol with alkyl halide (McMurry, 2004). However, etherification on phenolic compounds such as *p*-hydroxybenzaldehyde and *p*-hydroxyacetophenone is not as easy as those reported on aliphatic alcohols (Wright, 1997). The early example of etherification on *p*-hydroxybenzaldehyde was reported by Sugiyama *et al.* (1991) using 11-bromoundecanol, K₂CO₃ and KI in ethanol/water under refluxing condition. Many similar modified methods have also been reported (Yeap *et al.*, 2000; Ngaini, 2002; Cammidge *et al.*, 2003; Yeap *et al.*, 2004) which mainly involved bases such as KOH and K₂CO₃. However, problems may occur in *p*-hydroxyacetophenone due to the presence of α -hydrogen on the ketone. In the basic condition, the α -hydrogen on the ketone is prone to be activated by base, which subsequently gives an aldol product **1** (Scheme 1).



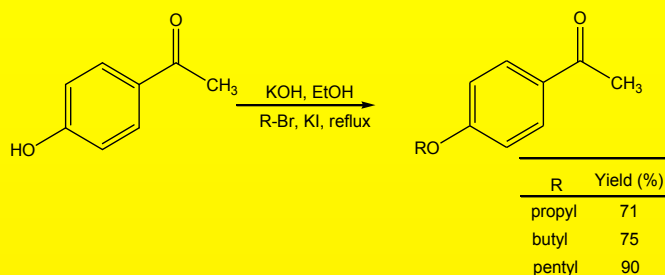
Scheme 1: Aldol-condensation of ketone

Indeed, Bhosale and his co-workers (2004) has reported a base-free catalytic method to etherify β -keto enol, and in most cases, they obtained excellent yields (>90%). Bhosale's method has been attempted in our early study for the etherification of *p*-hydroxyacetophenone but it was not successful. In 1997, Lai *et al.* reported a similar study on the etherification of *o,p*-dihydroxyacetophenone using K₂CO₃ and catalytic amount of KI under nitrogen atmosphere and refluxed in dried acetone for 24 hours to give 88% yield of the etherified product.

In this communication, we report a simple and convenient method for the etherification of *p*-hydroxyacetophenone under open air condition with the presence of KOH and KI in ethanol, with no aldol formation, to afford *p*-alkoxyacetophenones in 71 - 90% yields. We believed this method will help to synthesize alkoxychalcones, which have been reported concerning their biological properties (Liu *et al.*, 2001).

p-hydroxyacetophenone was added into a mixture solution of KOH and KI in ethanol and refluxed for 16 hours. The yields of the *p*-alkoxyacetophenones from the reaction of *p*-

hydroxyacetophenone with respective 1-propyl, 1-butyl and 1-pentyl bromide are shown in Scheme 2.



Scheme 2: Etherification of *p*-alkoxyacetophenones

IR spectrum showed the disappearance of OH⁻ peak for *p*-hydroxyacetophenone at 3166 cm⁻¹ and appearance of new peaks attributed to CH₃ asymmetric and symmetric vibrations at 2874, 2935 and 2960 cm⁻¹. The intensity of the peaks is depended on the length of an alkyl chain presence in the compounds; the longer alkyl chains the stronger intensity of the peak and vice versa (Furniss *et al.*, 1989).

Similarly, ¹H-NMR spectra of the three etherified products show the disappearance of OH⁻ signal at 10.10 ppm and the existence of new proton signals for the alkoxy group. This indicates the success of the etherification. It is important to note that [Ph-C(O)-CH₃, 3H] appeared as a singlet at 2.50 ppm in ¹H-NMR spectra of the three etherified products, which indicates that the α -hydrogens at the C(O)-CH₃ group were not affected by the presence of base in the reaction. We reasoned that the hydrogen at the phenolic group is more acidic, pK_a = 18.0 (Bordwell *et al.*, 1984) than the one at *p*-hydroxyacetophenone group, pK_a = 24.7 (Matthews *et al.*, 1975).

In conclusion, we have shown a convenient method for the etherification of *p*-hydroxyacetophenone without affecting the α -hydrogen at the acetophenone. The synthesis is straight forward and potentially offers advantages for the synthesis of chalcones and other organic compounds.

Acknowledgement

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Census survey of saltwater crocodile *Crocodylus porosus* in Batang Samarahan, Sarawak

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Saltwater crocodile, *Crocodylus porosus* in Sarawak is commonly referred as “Bujang Senang”. They live alongside the river and some of the habitats are located very near to human settlement. Besides the wide sluggish rivers and brackish swamps like the Batang Lupar and its tributaries, Sarawak mangrove areas, Batang Sadong and few other places, the *C. porosus* can also be found far up major rivers into very dissimilar habitats. Their hatchlings have been seen in the Loagan Bunut area, Sarawak's only natural lake which drains into Sungai Teru riverine system in the 4th Division (Cox & Gombek, 1985). The species was also reported to have been spotted at Upper Belaga and as far up the Kelauh, a small freshwater tributary of Batang Lupar. *C. porosus* have a unique feature compare to other species in family Crocodylidae as it is the only species without large scales on the back of its neck. It also has a large triangular head with broad and long snout and round eyes at the top of the head. *C. porosus* internationally have been listed in Appendix I in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), while in Malaysia protected by the Protection of Wildlife Act 1972 and Sarawak Wild Life Protection Ordinance 1998.

Increasing on crocodile attacks toward humans in major rivers in Sarawak have becoming concerning issue. These attacks trigger fears among people and have caused difficulties for them as many people in this state still depend on the rivers for foods, transportation and other activities. For example, crocodile attack occurred in Samarahan back in

December 2010 and the most recent attack occurred in Saratok (May 2011), both involved fatal attacks. In addition, crocodile attacks also provoke the villagers causing them to hold a grudge on crocodiles and sometimes foolishly taking matters into their own hands.

Patchy information on crocodile's population in Sarawak had caused difficulty in management of the human-crocodiles conflicts as well as carrying out sustainable management of the resource (crocodiles and crocodile-based products). Subsequently, a census survey had been carried out in Batang Samarahan and tributaries to assess the population density of *C. porosus*, hoping the data can be used to effectively manage the human-crocodile conflict in Sarawak.

Batang Samarahan and tributaries covers approximately 150 km long. The main river stretches out from Kampung Melayu in Samarahan to the river mouth. This river also connected to Sarawak River via Lobak Batu Belat tributary. Fishing is the main activity for people living alongside this river. In addition, this river also serves as mean of transportation including ferry and boat services. Others activities such as ongoing riverbank development, improved infrastructure development (roads and boat ramps) and logging activity also occur along this river.

Survey Methodologies

Census survey on crocodile populations in Batang Samarahan and tributaries was conducted from 17 January to 20 January 2011. Method used for this survey was night spotting technique modified from Cox and Gombek (1985), Games and Severre (1999) and Sullivan *et al.* (2010). On the boat, spotter scans riverbank or middle of the river for eyeshines, while paying close attention ahead for snags, rubbish and other hazards (Figure 1). Spotlight with more concentrate beam are better for wider scan of the eyeshines.



Figure 1: On boat, researchers scan riverbank or middle of the river for eyeshines using spotlight.

When the spotlight directed to the crocodiles, the retinal tapetum of a crocodile's eye will reflect distinctive orange or red colour (depending on the angle and intensity of lights). The light beam directed to the glowing eyes (Figure 2) often mesmerises the animal, allowing a close approach for capture or at least the spotter can approximate the size or age of the crocodiles (Figure 3).

When approaching the crocodiles, all participants of the survey remained silent and boat needed to slow (and sometimes glide according to river flow) to make sure that the crocodiles did not submerge in water.



Figure 2: As eyeshine spotted, Global Positioning System (GPS) reading was recorded. Data will be used for crocodiles mapping exercise.

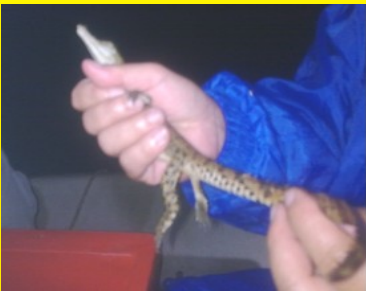


Figure 3: If possible, *C. porosus* was captured to estimate its size. When observers were unable to accurately estimate size class, the sightings were recorded as eyes only (EO).

During the survey, recordings were made of individual crocodile sightings as decimal degree, latitude and longitude waypoints using a Global Positioning System (GPS). All crocodiles were recorded according to the number of hatchlings, yearlings, sub-adults and adults. When observers were unable to accurately estimate size class, the sightings were recorded as eyes only (EO). The commencement location and end point of a survey was also recorded as a waypoint for the calculation of linear survey distance.

Results and Discussion

Altogether, 112 *C. porosus* were sighted from total of 140 kilometres of waterway surveyed (Figure 4), ranging from hatchling to adult (including ‘eyes only’). High number of hatchlings (34%) and relatively high number of ‘eyes only’ (28%) were recorded. ‘Eyes only’ usually refers to sub-adult and adult crocodiles, inhabiting mid-river, and usually submerge when the boat tried to approach them. Almost 10 % of the total numbers of *C. porosus* observed was sub-adult while 11% was adult. The mean relative density of non hatchling *C. porosus* recorded during these surveys was only 0.53 ± 0.34 (non-hatchlings/km). For comparison, Sullivan *et al.* (2010) reported that the density of crocodiles in Queensland, Australia was 0.49 ± 0.72 (non-hatchlings/km). Based on this preliminary finding, Batang Samarahan supports a balanced crocodile population in terms of size. The high number of hatchlings observed in this study suggests that successful nesting occurs along Batang Samarahan. The population bias is an indication of a recovering population (Sullivan *et al.*, 2010).

Based on observation during this survey, many ongoing riverbank development and improved infrastructure development (roads and boat ramps) along with logging activity had destroyed crocodile habitats. Therefore, increase of crocodile sightings by the human in this area is unavoidable. Incident of crocodile attack in December 2010 was the latest example of human-crocodile conflict occurs in Batang Samarahan.

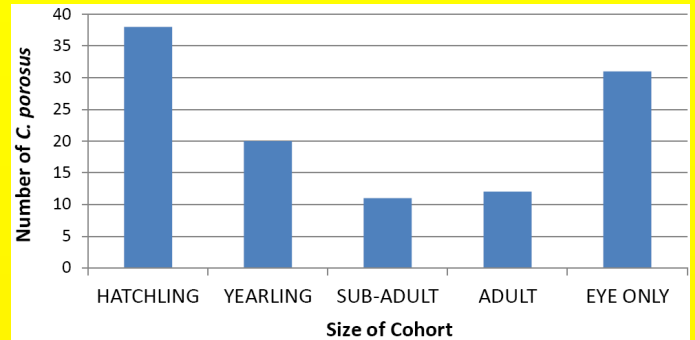


Figure 4: Number of *C. porosus* according to size of cohort recorded during the survey

There are many obstacles faced during the survey. According to Cox & Gombek (1985), surveys must be conducted during mid-low or low tide periods as the crocodiles are more concentrated in river and tributaries. Counting during high tide periods are significantly lower as crocodiles, especially small ones (hatchling and yearling) retires amongst flooded nipa and mangroves which more difficult to shines. The survey had been done prior to early low tide because the census of crocodiles (spot and count) need be done slowly. However, due to the enormous length of the river combined with fast flow of the tide into Batang Samarahan, the survey became relatively faster towards the end. Some parts of the counting exercise were done during high tide. Therefore, some crocodile might miss out during the counting.

Other problems faced during this survey were obstructing of light from bright moon or people’s house may interfere with counting exercise; in addition, as many villages and logging site build along Batang Samarahan, they contributed many snags, rubbish and other hazards to the river which make difficult for the spotter to differentiate light reflect between crocodile’s eye and rubbish. Cox & Gombek (1985) reported that conditions such as calm water, no rain or fog and no obstructing light from bright moon or others could increased the possibility to spot crocodile.

Conclusion

The population was strongly biased towards immature animals (hatchling and yearling) with 65 % of all *C. porosus* sighted. The population bias is indicative of a recovering population and the presence of hatchling indicating that there is some successful nesting occurring throughout Batang Samarahan. The mean relative density of non-hatchling *C. porosus* recorded during these surveys was only 0.53 ± 0.34 non-hatchlings/km. This survey was a part of ongoing study to monitor crocodile population along Batang Samarahan. Future works also include assessing the crocodile population structure of this area using microsatellite approach.

Acknowledgements

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In vitro cytotoxicity and antitermite activities of the essential oil of *Cinnamomum zeylanicum*

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In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals and insecticides. The chemical profile of the essential oil products can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage (Bakkali *et al.*, 2008). Essential oils contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts. Generally, the major components determine the biological properties of the essential oils (Bakkali *et al.*, 2008). Most of the chemical components of essential oils contained terpenoid compounds, including monoterpenes, sesquiterpenes, and their oxygenated derivatives. These low-molecular weight compounds present in the essential oils are easily diffuse across cell membranes to induce biological reactions (Kuan-Hung *et al.*, 2007).

The genus of *Cinnamomum* belongs to the Lauraceae family which consists of many species that yield volatile oil on distillation (Kuan-Hung *et al.*, 2007). The genus *Cinnamomum* is probably native from East and Southeast Asia to Australia (Wee and Hsuan, 1990). Some of the chemical constituents which are present in the essential oils of *Cinnamomum* species are very important due to their biological activity. The chemical constituents of the essential oils of *Cinnamomum* species gave a valuable effect especially in biological activities such as apoptosis-inducing activity of *Cinnamomum camphora* (Mehmet *et al.*, 2010), anticancer activity of *Cinnamomum cassia* (Mehmet *et al.*, 2010), gastroprotective activity of *Cinnamomum tamala* (Bavani *et al.*, 2010), antioxidative, insecticidal and anti-inflammation activities of *Cinnamomum osmophloeum* (Kuan-Hung *et al.*, 2007; Yu-Tang *et al.*, 2008; Sen-Sung *et al.*, 2009) and hypoglycemic activity of *Cinnamomum parthenoxylon* (Jiaa *et al.*, 2009).

The inner bark of *C. zeylanicum* has been used in ethno-medicine and flavoring for foods. Previous study showed that the essential oil of cinnamon bark exhibited potential antimicrobial and anticarcinogenic properties indicating the possibilities of its potential use in the formula of natural remedies for the topical treatment of infections and neoplasms (Mehmet *et al.*, 2010).

In the present study, the chemical compositions of the essential oil from the leaf of *C. zeylanicum* were identified and quantified. The biological activities of the leaf oil against brine shrimp larvae of *Artemia salina* and termites of *Coptotermes* sp. were evaluated. The results obtained can be used for further development of essential oil from this plant.

Chemical compositions of the leaf oil from *C. zeylanicum*

The essential oil from the leaf of *C. zeylanicum* was extracted using hydrodistillation method. The essential oil obtained was analyzed by Gas Chromatography-Mass Spectroscopy (GC/MS). The chemical compositions were identified by comparing their Kovat's retention indices with literature values and their mass spectral data with those from NIST mass spectral database. Only similarity indices higher than 85 were taken as proof of identity.

The percentage yield of essential oil obtained was 3.04% (vol/dry weight). The most abundant compounds found in the leaf oil of *C. zeylanicum* were eugenol and benzyl benzoate with amount of 72.76% and 14.39% respectively. Other chemical components present include eugenol acetate (1.96%), caryophyllene (1.58%), cinnamaldehyde (1.15%), caryophyllene oxide (0.93%), α -cubebene (0.79%), and cinnamyl acetate (0.72%) (Table 1 & Figure 1). In contrast with its bark oil, the important constituent were cinnamaldehyde (68.95%) and benzaldehyde (9.94%), cinnamyl acetate (7.44%), limonene (4.42%) and eugenol (2.77%) (Mehmet *et al.*, 2010).

Table 1: Chemical composition of the leaf oil from *C. zeylanicum*

Compounds	Kovat's Indices	Composition (%)
Cinnamaldehyde	1298	1.15
Eugenol	1375	72.76
α -Cubebene	1382	0.79
Caryophyllene	1428	1.58
Cinnamyl acetate	1464	0.72
Eugenol acetate	1531	1.96
Caryophyllene oxide	1598	0.93
Benzyl benzoate	1797	14.39

Cytotoxicity assay

Cytotoxicity assay of essential oil was carried out according to the method described by McLaughlin *et al.* (1998) against brine shrimp *Artemia salina* at 1.0 μ g/mL, 10.0 μ g/mL and 100.0 μ g/mL concentration. After 24 hours of contact, the number of survivors larvae in each well were counted and LC₅₀ values were determined.

The leaf oil of *C. zeylanicum* showed strong biological activity against the larvae of *Artemia salina* with LC₅₀ value of 10.0 μ g/mL. The high cytotoxicity of the essential oil exhibited by *C. zeylanicum* leaf towards brine shrimp,

Artemia salina indicated the potential of the oil as antitumor and pesticidal agents (Ishrak *et al.*, 2000).

Antitermite activity

The method established by Sakasegawa *et al.* (2003) was used for antitermite assay against *Coptotermes* sp. for 3 days at 1, 10 and 100 µg/mL concentration. The leaf oil showed inhibitory activity against the termites with 100% mortality after 3 days of treatment with LC₅₀ value of 63.10 µg/mL. The result showed the potential of the leaf oil for further development as environmental friendly termiticidal agent.

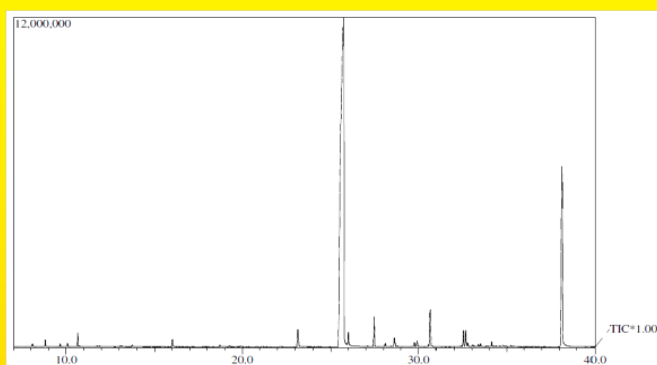


Figure 1: GC/MS Chromatogram for the leaf oil of *C. zeylanicum*

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The role of palaeofloras and Bornean aroids in the investigation of phytochores

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It was Edred J.H. Corner who first drew attention to the partial commonality of the tree floras of the E and NW coast of Peninsular Malaysia and that of N Borneo, and postulated this to be a result of these areas sharing remnants of a once extensive expanse of lowland humid forest and river deltas centred on what is now the South China Sea (Corner, 1960). Corner coined the name the 'Riau Pocket' for this, now fragmented, phytochore. Remarkably, for more than 40 years no-one picked up on Corner's landmark but much-overlooked paper. The intimation that interest was reviving in the exciting prospect of being able to study extant fragments of a "lost" biosphere joining the archipelagic and mainland elements of the Indomalaya Ecozone was a paper by Wong Khoo Meng (Wong, 1998) dealing with patterns plant endemism and rarity in Malaysia. Somewhat later still Peter Shaw Ashton (Ashton, 2005) mapped the known extent of the extant Riau Pocket fragments, and linked them to the known phytochores for Borneo. Between these papers, and subsequently, there have been a number of general and not a few specific studies drawing further reference to the importance of palaeobiogeography and modern distributions of taxa in SE Asia (van Welzen *et al.* 2003, Roos, 2004, van Welzen & Slik 2009, Raes *et al.* 2009), although none except Atkins *et al.* (2001), attempted phylogeographical analysis, and none has specifically analysed the veracity of the putative Riau Pocket phytochore fragments.

In order to attempt a phylogeographical study of disparate floristic fragments it is necessary to have a large and speciose taxon with an established phylogeny in which is combined deep (i.e., old) stem clades and recent, ideally species-rich, crown clades. Ideally too, the crown clades should include a high percentage of endemics together with a scattering on wide-spread species. The Araceae (aroid) flora of Borneo is universally acknowledged as one of the richest and most diverse on the planet, with 36 genera, of which 35 are indigenous, and one (*Typhonium* Schott) genuinely naturalized. Of the 35 indigenous genera, eight (*Aridarum* Ridl., *Bakoa* P.C.Boyce & S.Y.Wong, *Bucephalandra* Schott, *Ooia* S.Y.Wong & P.C.Boyce, *Pedicellarum* M.Hotta, *Phymatarum* M.Hotta, *Pichinia* S.Y.Wong & P.C.Boyce, and *Schottariella* P.C.Boyce & S.Y.Wong) are Bornean endemics. Currently there are 670 indigenous aroid species recorded for Borneo, of which more than 40% are undescribed novelties. Significantly this figure is based substantially on our understanding of the flora of Sarawak, Sabah and to a much lesser degree Brunei (i.e., less than one third of the total landmass). Kalimantan, comprising more than 70% of the land area of Borneo remains very poorly known, and undoubtedly harbours a great many novel species. It is estimated that the total aroid flora for Borneo quite likely exceeds 1000 species, with barely one third of these described. Borneo is thus an aroid habitat of global significance, and arguably one of the richest and diverse on the planet.

One of the largest and most diverse aroid taxa on Borneo is the Schismatoglottideae, a robustly monophyletic clade sister to Cryptocoryneae that, together Neotropical *Philonotus*, comprises the Schismatoglottid Alliance. Species of Schismatoglottideae are in the main adapted to riverine humid lowland forest ecologies, notably gallery forest and particularly in Borneo often occurring as obligate or facultative rheophytes. Many Schismatoglottideae species are of highly restricted distribution, often on particular geologies and not infrequently occurring allopatrically and parapatrically as 'guilds' of morphologically similar species.

Molecular and palaeo-eco-geographical data support that the Schismatoglottid Alliance is very old, with diversification of the Neotropical and Palaeotropical clades pre-dating the break-up of the southern supercontinent of Gondwana (ca 120 MYA). Stem clades are in the main somewhat more recent, although the deepest (e.g., *Apoballis*) are doubtless nearly contemporary to the preliminary Neo-Paleotropical separation. By comparison, evidence suggests that most crown clades are of recent separation/radiation, in some instances (e.g., those present in the Lambir Formation) perhaps less than 20KA, although most are more likely diversifications dating from 2–4 MYA. This combination of an ancient origin with much more recent diversification makes analyses of Schismatoglottideae a potent tool to investigate the medium term to recent origins of the mesophyte biota of the perhumid forests of Sunda and thus gain understanding of the occurrence and demarcation of phytochores. Preliminary evidence is that much of the most recent species diversification coincides with times of low sea levels in Sunda that were occasioned by periods of glacial maxima in the northern hemisphere.

It is still early days in the study, but already it is clear that the modern Schismatoglottideae flora of Borneo results from a mosaic of evolutionary radiations occurring in a series of pulses, with the earliest >2MYA, and the most recent probably <20KYA. The Bornean Riau Pocket fragments postulated (based on tree floras) by Ashton are broadly supported and exemplified by the presence of *Phymatarum* M.Hotta (Figure 1) in the east and a profuse assemblage of taxa in the *Schismatoglottis multiflora* and *S. asperata* complexes in the NW, together with *Aridarum* Ridl. sect. *Aridarum*, and the Elongata complex of *Piptospatha* N.E.Br. The Lupar Line is maintained, although in a somewhat modified form, with its southern extremity now terminating above the Ai river, and with the area to the E and SE of this termination marking the edge of a new phytochore along the Sungai Sarikei and its associated catchments and probably extending through the saddle of the Kapuas Hulu to the Muller and Schwaner ranges, and extending to the Kapit side of the Sungai Song. This new phytochore is exemplified by the genera *Schottariella* (Figure 2) and *Schottarum* (Figure 3). Predictably the cohesiveness of the Rejang drainages (i.e., east of the Sungai Song and west of the Balui drainages /Hose Mountains) is well-supported, with signature Schismatoglottideae being *Aridarum* sect. *Caulescentia* M.Hotta (Figure 4), among others. With the possible exception of links to Niah and Bukit Satiam (Bintulu) the Mulu

phytochore seems independent of the main Sarawak aroid flora and instead is allied, via the Setap Shales to that of SW Brunei. Typical of the Mulu phytochore are species allied to *Schismatoglottis dilecta* S.Y.Wong, P.C.Boyce & S.L.Low (Figure 5).



Figure 1: *Phymatarum borneense* M.Hotta



Figure 2: *Schottariella mirifica* P.C.Boyce & S.Y.Wong



Figure 3: *Schottarum sarikeense* (Bogner & M.Hotta) P.C.Boyce & S.Y.Wong



Figure 4: *Aridarum caulescens* M.Hotta



Figure 5: *Schismatoglottis dilecta* S.Y.Wong, P.C.Boyce & S.L.Low

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constructive criticisms from the participants as well as non-participants had motivated the organizer to further improve future JSCU and most likely JSCU will be FRST yearly-event.



Science requires accurate measurement... practice makes perfect!



Enjoyable moments trapping the insects using light trapping technique.



Just like school children, soil particles are small but strong!



Plants do not need soil to grow?



Small mammals are not scary monsters; they are actually our interesting cousins!



Who is going to be the next Professor?

Junior Science Camp UNIMAS 2011: 'Learning SCIENCE IS FUN'

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¹Department of Zoology
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The Faculty of Resource Science and Technology (FRST), Universiti Malaysia Sarawak (UNIMAS) has successfully organized the Junior Science Camp UNIMAS 2011 (JSCU 2011) on the 9th and 10th April 2011 at the External Laboratory, FRST, UNIMAS. This two-day camp was designed to nurture interest among young budding scientists of tomorrow by giving them opportunities to experience life as science university students on day-to-day basis. The programme has also allowed the faculty to outreach the community outside UNIMAS, by promoting academic programmes offered by the faculty besides raising awareness on local flora and fauna diversity and other science-related matters.

JSCU 2011 involved 60 primary six students and 4 teachers from SK Green Road Kuching supported by 75 staffs and graduate students of FRST. The carefully-planned activities of JSCU were based on school curriculum, combining hands-on laboratory sessions (Aquatic Science, Animal Science, Chemistry, Biotechnology, Plant Science and Mathematics), field work and motivation talk, as below:

No.	Title	Resource Person(s)
A. Laboratory sessions		
1.	Aquatic Science and Daily Life	Dr. Siti Akmar & Dr. Ruhana
2.	Animal Science and Diversity	Dr. Ramlah & Dr. Yuzine
3.	Chemistry	Dr. Chin Suk Fun
4.	Biotechnology in the Kitchen	Dr. Azham Zulkharnain
5.	Plant and Soil Properties	Dr. Effendi Wasli, Mr. Abas Said & Ms. Morgeret Sidi
6.	Playing with numbers – The Power of Prediction	Mr. Charlie Laman, Ms. Siti Nur Lydia & Mr. Mohd Nasarudin
B. Field Experience		
1	Frogging and Insect trapping	Dr. Ramlah, Mr. Wahap Marni & Mr. Mohd Jalani Mortada
2	Fun in Bird Watching	Dr. Lim Chan Koon & Ms. Zahrunisa Abd Rahim
C. Motivational Talk		
1	Past, Now and Future Kids	Prof. Dr. Mustafa Abdul Rahman
2	Road to Professorship (Entomology)	Prof. Dr. Sulaiman Hanapi

Based on survey carried out at the end of JSCU 2011, majority of the participants enjoyed themselves and value the experiences gained. Positive feedback and several

Development and characterization of expressed sequence tag-simple sequence repeat markers from Kelampayan tree transcriptome (NcdbEST)

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Expressed sequence tag-simple sequence repeats (EST-SSRs) are a small array of tandemly arranged bases (1-6 bp) which embedded in functional gene sequences which may lead to the identification of genes controlling these traits. The variation in the SSR lengths occurs primarily due to the mutational effects of replication slippage and unequal recombinant. Thus, EST-SSR marker has the ability to reveal polymorphism than do most other genetic markers. The diversity analysis using EST-SSR markers has greatly facilitated the genetic improvement as well as *ex situ* conservation strategies of plant genetic resources (Feng *et al.*, 2009).

Neolamarckia cadamba (Roxb.) Bosser, locally known as Kelampayan belongs to the family of Rubiaceae (**Figure 1**). It is praised as a “miracle tree” in China due to its fast growing characteristics and an ideal tree species to study genetic functions related to tree growth and cell wall development (Li *et al.*, 2011). Kelampayan has been selected as one of the fast growing plantation species for planted forest development in Sarawak. It is one of the best sources of raw material for the plywood industry, besides pulp and paper production. It also has high potential to be utilized as one of the renewable resource of raw materials for bioenergy production such as cellulosic biofuels in the near future.



Figure 1: The current and other/future uses of Kelampayan

As of May 2010, total area planted with Kelampayan was 18,851 ha (8% out of 240,075 total area planted) in Sarawak, which is approximately about 9 million trees planted with planting distance of 4 x 4 meters. Thus, the development of tools which facilitate the selection of quality planting material was greatly needed.

In this study, 155 sequences which contain 231 SSRs were identified from 6,622 ESTs obtained from Kelampayan tree transcriptome (NcdbEST). Of these, 57 (36.77%) EST-SSRs

did not show any significant match to the NCBI non-redundant protein database (E-value < 1e-15), whereas 98 (63.23%) were assigned with putative functions. Gene Ontology (GO) terms were further assigned to the sequences that were successfully annotated. 91 (58.71%) were able to be assigned with GO numbers. Primary metabolic process (16.67%) is the most dominant group that was annotated to the biological process category. It is followed by the cellular metabolic process at 13.33%, macromolecule metabolic process at 11.67% and biosynthetic process at 10.56%. Nucleic acid binding (33.33%) is the most dominant group in molecular function category, followed by hydrolase activity (20.37%) and protein binding (18.52%). With regard to the cellular component, 25% are assigned to plastid followed by nucleus (22%) and mitochondrion (16%).

Among all 231 SSRs identified from 155 ESTs, tri-nucleotide SSR is the dominant repeat type (56.31%), followed by dinucleotide (22.82%), tetra-nucleotide (5.83%), hexa-nucleotide (2.43%), penta-nucleotide (0.97%), perfect compound (1.94%) and imperfect compound (9.71%) SSR. Nineteen out of 24 EST-SSR primers designed exhibit clear and single locus amplification patterns were further used for polymorphism screening from 52 Kelampayan trees (half-sib family) which derived from 2 different mother trees (25 seedlings each). The fragment sizes were ranging from approximately 90 bp to 500 bp. The number of alleles ranged from 1 (NCS16) to 13 (NCS05). The mean value of polymorphism information content (PIC) for mother tree 1 and 2 was 0.470 and 0.549, respectively. The markers show PIC value up to 0.876 (NCS05) which is higher compared to *Coffea* species with PIC value 0.750. In general, the samples used in this study generate considerably high level of genetic diversity (mean P = 84.48%, $A_a = 4.368$, $A_e = 3.508$, $H_o = 0.225$, $H_e = 0.561$) (**Table 1**). These results indicate that the EST-SSR markers used in this study were informative and can be used for genetic and breeding studies in Kelampayan.

Table 1: Summary of genetic diversity parameters estimated for Kelampayan trees (half-sib family)

Mother tree	N	P (no. P)	PIC	A_a	A_e	Mean heterozygosity	
						H_o	H_e
1	26	84.2% (16)	0.470	4.26	2.783	0.209	0.520
2	26	94.7% (18)	0.549	4.47	3.332	0.242	0.602
Mean	26	89.5% (17)	0.506	4.37	3.058	0.225	0.561

In conclusion, EST-SSR markers developed in this study will be the valuable resource for functional diversity analysis in Kelampayan to exploit the genotyping data for tree improvement as well as conservation of plant genetic resources. These markers will facilitate the selection of quality planting material, as well as the saving in time and cost for the planted forest development in Sarawak.

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FRST's Upcoming Events

The 2nd International Symposium on Eco-Zoonoses and Emerging Infectious Diseases, 14-16 December 2011, CAIS Auditorium, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak

Next Generation Sequencing Technologies Workshop 2012 (NGST2012) with special focus on Molecular Breeding of Plant and Animal, 13-14 September 2012, jointly organized by the Faculty of Resource Science and Technology, UNIMAS, Sarawak and Beijing Genomics Institute (BGI), Shenzhen-China

Conference on the Natural Resources in the Tropics 4 (NRTROP4), 17-19 September 2012, Hilton Hotel, Kuching, Sarawak



International Conference and Exposition on Inventions of Institutions of Higher Learning
Invigorating Innovation in the New Economic Model

A total of 25 researchers from UNIMAS recently participated at the International Exposition of Research and Invention of Institutions of Higher Learning 2011 (PECEIPTA). The exposition which was held from 13th to 15th September 2011 was organized jointly by the Ministry of Higher Education and Universiti Teknologi Mara at the Kuala Lumpur Convention Centre (KLCC). The objective of PECEIPTA 2011 is to bridge innovation from academia to the business industry. Two projects by FRST won Bronze Medal. The projects are:

1. Efficacy of new Organotin(IV) compounds against wood decay fungi

by Assoc. Prof. Dr. M.A. Affan, Md. Abdus Salam, Prof. Dr. Fasihuddin B Ahmad, Assoc. Prof. Dr. Ismail B Jusoh and Dr. Md. Rezaur Rahman

2. fasTip-X Kit

by Lai Pei Sing, Dr. Ho Wei Seng, Dr. Pang Shek Ling and Assoc. Prof. Dr. Ismail B Jusoh

FRST's Recent Publications

- Affan, M.A., Salam, M.A., Fasihuddin, B.A., Ismail, J., Mustaffa, B.S. and Hapipah, M.A. (2011). Synthesis and spectroscopic characterization of organotin(IV) complexes with 2-benzopyridine-N(4)-cyclohexylthiosemicarbazone (HBPCT): X-ray crystal structure of [PhSnCl₂(BPCT)]. *Inorganica Chimica Acta* 366: 227-232.
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