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# Volatile Oil Composition of *Neobalanocarpus heimii*

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*Neobalanocarpus heimii* locally known as 'chengal' is one of the most durable tropical timber species found in Malaysia. The tree occasionally produce flowers and when it does it gives out some scented fragrance to the surrounding. In this study, the flowers of *N. heimii* were collected, distilled and examined by automated HS-SPME-GCMS and GCMS. The volatile constituents consisted of β-caryophyllene (25.02%), germacrene D (13.02%), β-bisabolene (9.02%), (*2E,6Z*)-farnesol (7.56%), α-humulene (6.18%), β-sesquiphellandrene (4.25%), bicyclogermacrene (3.06%), 7-*epi*-sesquithujene (2.64%), α-bisabolol (1.67%), caryophyllene oxide (1.50%), α-cadinol (1.11%) and (*Z,E*)-α-farnesene (0.98%). All HS-SPME extractions were conducted using PDMS and DVB/CAR/PDMS fibres and showed similar typical chromatograms as in essential oils but with varying concentrations. Chemical compounds which might be responsible for the aroma of the flower oils could be described by the presence of (*E*)-β-farnesene, β-caryophyllene, β-bisabolene and *cis*-α-bergamotene.

**Key words:** *Neobalanocarpus heimii*; *chengal*, HS-SPME; β-caryophyllene; β-bisabolene

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*Neobalanocarpus* is a monotypic genus in the family Dipterocarpaceae. It is estimated that 16 genera and more than 500 species occur in the same family such as *Shorea*, *Hopea*, *Dipterocarpus*, *Anisoptera* and *Vatica* [1]. Saw and Sam [2] reported that, Dipterocarpaceae comprises 157 species in Peninsular Malaysia. *Neobalanocarpus heimii* locally known as 'chengal' is a tropical hardwood tree that has been used as the wood of choice for house and boat building in Peninsular Malaysia and sometimes referred to as the 'Malaysian Teak'. The properties of this species have been the subject of much researches which include timber performance, phenological observation, DNA profiling and biological activities.

Many studies were carried out on the chemical composition of various species from Dipterocarpaceae mainly on the oligostibenes and resveratrol oligomers. Previous study reported that genus of *Shorea* contained oligostilbene [3,4], isohopeaphenol [4–7], balanocarpol [4] and vaticanol A [4,8]. The essential oil composition of *Shorea robusta* and *S. acuminata* was also been studied. The main components reported were t-cadinol (16.8%) and α-cadinol (16.5%). Caryophyllene oxide (36.0%) and β-caryophyllene (13.9%) were the main components of *Shorea acuminata* leaf oil. Germacrene D was detected as the major component of stem oils [9].

Recent investigations on *Neobalanocarpus heimii* reported that this species have interesting biological properties probably due to the presence of phenolic compounds and oligostilbenes [10]. The ethyl acetate extracts of *N. heimii* show fungicidal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* [11]. Kawamura *et al.* [2] reported that the methanol extracts from the bark have moderate antifungal activity against a brown-rot fungus, *Gloeophyllum trabeum* and strong antifungal activity against a white-rot fungus, *Pycnoporus*

*sanguineus*, at a minimum effective amount of 100 µg. According to Roszaini *et al*. [3], wood and bark essential oils and extracts of *N. heimii* have promising anti-termitic properties against subterranean termites. Some of the chemical compounds detected are benzyl carbinol, benzyl isoamyl ether, eicosane, cyclopentanone and farnesol. Higher concentration of extracts from the bark and hard wood of *N. Heimii* gave 100% mortality of 3rd instars of *Coptotermes* gestroi. The activities of these extracts were equal to the activity of the positive control, glycyrrhizic acid dipotassium salt, suggesting that they have great potential as a source of fungistats.

Twenty years of monthly phenological observations on this species at Forest Research Institute Malaysia (FRIM), Pasoh Forest Reserve (Pasoh FR), Ulu Gombak Forest Reserve (Ulu Gombak FR) and Ampang Forest Reserve (Ampang FR) indicate that there is a high probability (>95%) of an onset of flowering following the end of a dry period. Flowering occurs in most years (either annually or biannually). The event can either peak during March to May and maybe followed by a second peak during September until November [14]. The flowers of *N. heimeii* has a fine and sweet jasmine-like smell which disperses to the surrounding. In order to trap the volatile part from this flower, headspace-solidphase micro-extraction (HS-SPME) extraction can be used. Previous studies have reported the uses of HS-SPME coupled with GCMS for identification of volatile constituents of fresh flowers [15,16]. In the year 2014, the flowering event was observed to occur between March and May. Since there is no previous report on flower essential oils from *N. heimii,* we took the opportunity to study the volatile constituents of the flowers in this paper.

## EXPERIMENTAL

### Plant Sample and Preparation

The fresh flowers of *Neobalanocarpus heimii* were collected in the early morning from a tree in the FRIM campus. The small flowers (200 g) were immediately water distilled for 6 h and the oily layers obtained were separated and dried over anhydrous sodium sulphate. The yields were averaged over two experiments and calculated on a dry weight basis of the plant material. For solid-phase micro extraction, 2 g fresh flowers were placed into 20 ml screw-top clear vial, tightly capped and allowed to equilibrate for 10 min at room temperature prior to HS-SPME-GCMS experiments.

### Chemical Analysis

Quantitative analysis of the flower oil was carried out using Shimadzu GC 2010 and Agilent Technologies GCMS 7890A/5975C Series MSD apparatus equipped with fused silica capillary columns CBP5 (25 m × 0.25 mm, 0.25 mm film thickness) and HP-5MS column (30 m × 0.25 mm ID × 0.25 mm film thickness) for GC and GCMS, respectively. The gas chromatography was equipped with FID using split mode injection technique and the operating parameters were helium gas as carrier gas at a flow rate of 1 ml/min, injector temperature 250°C, and detector temperature 250°C. With the CBP5 column, the gas chromatography was programmed initially at 60°C for 10 min, then to 230°C for 1 min at 3°C/min. For GCMS analysis, the temperature programme was set similarly to GC programme. The chemical constituents were identified by mass spectral library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L). The results of the peak areas were expressed as peak area counts.

### HS-SPME-GCMS

HS-SPME-GCMS analyses were performed by using a CombiPaL autosampler which was attached to Agilent Technologies 7890A/5975C Series MSD. Two fibres viz. Stableflex™ fibre of 100 mm polydimethylsiloxane (PDMS) and Stableflex™ fibre of 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) were purchased from Supelco company and conditioned individually in the fibre conditioning station for 20 min at 250°C and cleaned between analyses to avoid contamination. The incubation temperature that was used for this study was 50°C. The extraction time was fixed at 15 min to enable volatiles to be trapped. After sampling, the fibre was immediately inserted into the GC injector for 5 min and were analysed using HP-5MS (30 m × 250 µm × 0.25 µm) and programmed at 60°C for 10 min then 3°C/min to 180°C for

1 min. The chemical constituents were identified by comparison of their mass spectrum with the mass spectral library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L).

## RESULTS AND DISCUSSION

Table 1 lists the oil constituents identified in the flower oil of *Neobalanocarpus heimii.* Through hydrodistillation, the essential oil of the fresh flowers was yellow in colour and yielded 0.15% (w/w), based on the dry weight of plant sample. Analyses by GC and GCMS showed that a total of 18 compounds amounting of 79.2% in the essential oil were identified. The volatile constituents were made up of sesquiterpenoids, its oxygenated derivatives and sesquiterpenes hydrocarbon. β-Caryophyllene (25.02%), α-humulene (6.18%), (*Z,E*)-α-farnesene (0.98%), germacrene D (13.02%), β-bisabolene (9.02%), caryophyllene oxide (1.50%), α-cardinol (1.11%), α-bisabolol (1.67%), and (*2E,6Z*)-farnesol (7.56%) were some of the chemical compounds detected in the flower oils.

Solid phase microextraction (SPME) is currently an extraction method of choice particularly for analysing volatile samples from smaller amounts of samples within a shorter time and solvent free. PDMS and DVB/CAR/PDMS fibres were chosen due to its ability to extract and trap volatiles from aromatic samples. From our study, the HS-SPME-GCMS analysis conducted showed that the volatile components were detected by the presence of sesquiterpenoids; (*E,E*)-α-farnesene, α-copaene, α-gurjunene, α-cubebene, β-caryophyllene, α-ylangene, *cis*-α-bergamotene, γ-muurolene, (*E*)-β-farnesene, germacrene D, β-bisabolene and β-sesquiphellandrene as shown in Table 2.

All HS-SPME extractions conducted using both fibres showed similar typical chromatograms but with varying concentrations but failed to detect the presence of aroma compounds such as (*Z,E*)-α-farnesene, α-bisabolol and (*2E,6Z*)-farnesol. The results also showed that compounds such as α-cubebene, α-ylangene and γ-muurolene were

**Table 1.** Percentage compositions of the volatile constituents of *Neobalanocarpus heimii* flower oil.

| No | Compound | $KI_{cal}$ | Average Area (%) |
|---|---|---|---|
| 1. | α-copaene | 1363 | 0.82 |
| 2. | 7-*epi*-sesquithujene | 1385 | 2.64 |
| 3. | α-gurjunene | 1396 | 0.37 |
| 4. | β-caryophyllene | 1423 | 25.02 |
| 5. | (*Z,E*)-α-farnesene | 1425 | 0.98 |
| 6. | α-humulene | 1455 | 6.18 |
| 7. | Germacrene D | 1489 | 13.02 |
| 8. | Bicyclogermacrene | 1498 | 3.06 |
| 9. | β-bisabolene | 1518 | 9.02 |
| 10. | β-sesquiphellandrene | 1531 | 4.25 |
| 11. | α-bisabolene | 1543 | 0.06 |
| 12. | Nerolidol | 1554 | 0.76 |
| 13. | Caryophyllene oxide | 1573 | 1.50 |
| 14. | Viridiflorene | 1596 | 0.42 |
| 15. | Selina-3,7(11)-diene | 1632 | 0.56 |
| 16. | α-cadinol | 1658 | 1.11 |
| 17. | α-bisabolol | 1700 | 1.67 |
| 18. | (*2E,6Z*)-farnesol | 1727 | 7.56 |
| 19. | (*6S,7R*)-bisabolene | 1762 | 0.15 |

Note: $KI_{cal}$ = Calculated Kovat index (compound are listed in order of their elution from a CBP5 column)

**Table 2.** Major chemical constituents from rapid chemical analysis using HS-SPME-GCMS for *Neobalanocarpus heimii*

| No | Compound | Average area (%) | |
|---|---|---|---|
| | | PDMS | DVB/CAR/PDMS |
| 1. | α-cubebene | t | 0.51 |
| 2. | α-ylangene | t | 0.19 |
| 3. | α-copaene | 1.55 | 2.51 |
| 4. | α-gurjunene | 0.41 | 1.20 |
| 5. | *cis*-α-bergamotene | 3.59 | 2.74 |
| 6. | β-caryophyllene | 24.18 | 18.95 |
| 7. | *trans*-α-bergamotene | 2.88 | 1.98 |
| 8. | (*E*)-β-farnesene | 17.72 | 14.52 |
| 9. | γ-muurolene | 0.38 | 5.56 |
| 10. | Germacrene D | 10.01 | 0.95 |
| 11. | β-bisabolene | 13.59 | 11.16 |
| 12. | β-sesquiphellandrene | 4.84 | t |

Note: t = trace

selectively detected using a DVB/CAR/PDMS fibre whereas PDMS fibre was able to detect more of germacrene D and β-sesquiphellandrene.

The aroma chemistry of flowers are complex and is never made up of single chemical compound. However, the presence of specific chemical compounds or molecules in the oils may give an impart towards the flower aroma. Therefore from this study, chemical compounds which might be contributing to the sweet faint aroma of the chengal flower oils could be described by the presence (*E*)-β-farnesene, β-caryophyllene, β-bisabolene and *cis*-α-bergamotene. These compounds are similarly found in other flower oils such as ylang –ylang and jasmine oils [17,18].

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