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# Essential oil composition of three *Cryptocarya* species from Malaysia

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Abstract: Cryptocarya species are mainly distributed in Africa, Asia, Australia and South America, widely used in traditional medicines for the treatment of skin infections and diarrhea. The present investigation reports on the extraction by hydrodistillation and the chemical composition of three Cryptocarya species (Cryptocarya impressa, Cryptocarya infectoria, and Cryptocarya rugulosa) essential oils from Malaysia. The chemical composition of these essential oils was fully characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of 51 components were identified in C. impressa, C. infectoria, and C. rugulosa essential oils representing 91.6, 91.4, and 83.0% of the total oil, respectively. The high percentages of  $\alpha$ -cadinol (40.7%) and 1,10-di-epi-cubenol (13.4%) were found in C. impressa oil. β-Caryophyllene (25.4%) and bicyclogermacrene (15.2%) were predominate in *C. infectoria* oil. While in *C. rugulosa* oil, bicyclogermacrene (15.6%),  $\delta$ -cadinene (13.8%), and  $\alpha$ -copaene (12.3%) were predominate. To the best of our knowledge, there is no report on the essential oil composition of these three species.

**Keywords:** *Cryptocarya impressa*; *Cryptocarya infectoria*; *Cryptocarya rugulosa*; essential oil; GC-MS; Lauraceae.

## 1 Introduction

Lauraceae comprises about 55 genera and over 2500 species mostly found in tropical, subtropical and mild temperate regions. Due to their commercial importance, some

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Lauraceae species have already been studied regarding their essential oil contents and biological activities. The largest of the early-diverging clades within the Lauraceae family is the Cryptocarya genus which comprises around 350 plant species distributed across Africa, Asia, Australia, and South America, in which 19 species have been found in Malaysia [1]. Many plant species from this genus have been widely used in traditional medicines for the treatment of skin infections and diarrhea [2]. Additionally, several phytochemical studies have been undertaken on this genus, resulting in the identification of a significant number of natural products such as flavonoids [3], α-pyrones [4], alkaloids [5], lignans [6], and phenylpropanoids [7] in addition to diverse biological activities including anti-inflammatory [8], antiplasmodial [9], anti-tuberculosis [10], antimalarial [11], and cytotoxic [6] activities.

Although many members of the family Lauraceae are renowned for their valuable essential oils, the genus *Cryptocarya* is still poorly explored as far as its essential oil composition is concerned. Therefore, it is interesting to report the essential oil composition of three *Cryptocarya* species (*Cryptocarya impressa*, *Cryptocarya infectoria*, and *Cryptocarya rugulosa*). This is the first study of its kind to investigate the chemical composition of the essential oils extracted from these species found in Malaysia.

## 2 Materials and methods

#### 2.1 Plant material

The fresh samples of *C. impressa* (SK03/16) and *C. rugulosa* (SK04/16) were collected in October 2016 from Kluang, Johor, while *C. infectoria* (SK389/19) was collected from Behrang, Perak in January 2019. All samples were identified by Dr. Shamsul Khamis, a botanist from Universiti Kebangsaan Malaysia (UKM). The voucher specimens were deposited at UKMB Herbarium, Faculty of Science and Technology at UKM.

#### 2.2 Extraction of essential oils

The fresh leaves of each sample (500 g) chopped into small pieces and continue subjected to hydrodistillation process in Clevenger-type

apparatus for 4 h. The essential oils obtained were then dried over anhydrous magnesium sulfate and stored at  $4-6\,^{\circ}$ C. The oil yield (%) is calculated based on the fresh weight (w/w).

#### 2.3 Analysis of essential oils

GC-FID analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with HP-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280 °C, respectively. The oven temperature was kept at 50 °C, then gradually raised to 280 °C at 5 °C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with HP-5 column. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at 10 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50–400 amu.

#### 2.4 Identification of chemical components

For identification of the essential oil components, co-injection with the standards (major components) were used, together with correspondence of retention indices (relative to the retention times of n-alkanes from  $C_6$  to  $C_{30}$ ) and mass spectra with respect to those reported in Adams, NIST08, and FFNSC2 libraries [12]. Semi-quantification of the essential oil components was undertaken by peak area normalization considering the same response factor for all volatile components. Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

## 3 Results and discussion

The hydrodistillation of the leaves of *C. impressa*, *C. infectoria*, and *C. rugulosa* yielded pale yellow oils having a pungent odour in the mean yields of 0.35, 0.34, and 0.38% (w/w), respectively. The GC and GC-MS analyses successfully identified 51 components as presented in Table 1. Here, sesquiterpene hydrocarbons were identified as the major group components in *C. infectoria* and *C. rugulosa* oils which constituted 72.7 and 68.4%, respectively, while *C. impressa* oil dominated with oxygenated sesquiterpenes accounting for 54.2%. However, monoterpenoids were not discovered in all essential oils.

A total of 27 components were identified from the essential oil of *C. impressa*, representing 91.6% of the total

oil. The most substantial components were  $\alpha$ -cadinol (40.7%), 1,10-di-epi-cubenol (13.4%), germacrene D (7.2%), and y-cadinene (5.5%), while form the essential oil of C. infectoria, 28 components were identified (91.4%) of which βcaryophyllene (25.4%), bicyclogermacrene (15.2%), germacrene D (9.9%),  $\delta$ -cadinene (7.0%), and globulol (5.1%) represented the major components. In addition, the essential oil of C. rugulosa yielded 29 components, which represented 83.0% of the total oil, with bicyclogermacrene (15.6%),  $\delta$ cadinene (13.8%), α-copaene (12.3%), and aromadendrene (5.6%) identified as the most abundant components. Based on the above studies, 15 components (safrole, methyl eugenol, β-copaene, ishwarane, amorpha-4,7(11)-diene, eudesma-6,11-diene, *cis*-eudesma-6,11-diene, (E)-methyl isoeugenol, viridiflorene, premnaspirodiene, v-cadinene, trans-cadina-1,4-diene, β-calacorene, 1,10-di-epi-cubenol, and shyobunol) were only discovered in C. impressa oil, whereas six components (isoledene, longifolene, globulol, junenol, 1-epi-cubenol, and epi-α-muurolol) were only identified in C. infectoria oil. Meanwhile, seven components (β-bourbonene, β-cubebene, β-selinene, cis-cadina-1,4diene, α-calacorene, elemol, and cubenol) were only found in C. rugulosa oil. Moreover, 10 components ( $\alpha$ -copaene,  $\beta$ elemene, β-caryophyllene, aromadendrene, α-humulene, germacrene D,  $\delta$ -cadinene,  $\alpha$ -cadinene, germacrene B and  $\alpha$ cadinol) were discovered in all essential oil fractions of the three plant samples under investigation. Besides, safrole was the only phenylpropanoid present in C. impressa oil. The compound was found to be toxic and a weak hepatocarcinogen by the US Food and Drug Administration (FDA) [13].

Of these, bicyclogermacrene was found as the major components in C. infectoria and C. rugulosa essential oils previously reported as the principal components in the leaves oil of Cryptocarya cocosoides (25.5%) and Cryptocarya lividula (26.1%) [14]. On the other hand, benzyl benzoate has been identified from the fruit's oil of Cryptocarya massoy (68.0%) [15] and the leaves oil of Cryptocarya cunninghamii (80.2%) [14]. Also, the bark/heartwood oil of C. massoy, 5,6-dihydro-6-pentyl-2H-pyran-2-one (64.8-68.4%), was reported as being the most abundant component [16]. Several studies have reported monoterpenes as the foremost components from the leaves oil of Cryptocarya aschersoniana (limonene 42.3%) [14] and *Cryptocarva bellendenkerana* (β-phellandrene 11.8%) [14]. However, in this study, no monoterpenes were found in all Cryptocarya essential oils. The chemical differences among Cryptocarya species could be due to the extraction procedures, stages of development, and distinct habitat in which the plant was collected [17]. Besides, the chemical and biological diversity of aromatic and medicinal plants depend on such factors as climatic conditions, vegetation

**Table 1:** Chemical components identified from *Cryptocarya* essential oils.

| No | Components                 | KIª  | ΚI <sup>b</sup> |                                 | Methods <sup>d</sup>            |                                 |             |
|----|----------------------------|------|-----------------|---------------------------------|---------------------------------|---------------------------------|-------------|
|    |                            |      |                 | CIPO                            | CIFO                            | CRGO                            |             |
| 1  | Safrole                    | 1282 | 1285            | 0.6 ± 0.2                       | _                               | _                               | RI, MS      |
| 2  | δ-Elemene                  | 1334 | 1335            | $\textbf{0.4} \pm \textbf{0.2}$ | _                               | $0.3 \pm 0.2$                   | RI, MS      |
| 3  | α-Cubebene                 | 1340 | 1345            | _                               | $0.2\pm0.1$                     | $2.5\pm0.3$                     | RI, MS      |
| 4  | Isoledene                  | 1375 | 1374            | _                               | $0.6\pm0.1$                     | _                               | RI, MS      |
| 5  | α-Copaene                  | 1376 | 1374            | $0.8\pm0.2$                     | $\textbf{1.5} \pm \textbf{0.2}$ | $12.3\pm0.2$                    | RI, MS, Std |
| 6  | β-Bourbonene               | 1385 | 1387            | _                               | _                               | $\textbf{0.5} \pm \textbf{0.1}$ | RI, MS      |
| 7  | β-Cubebene                 | 1386 | 1387            | _                               | _                               | $\textbf{1.0} \pm \textbf{0.2}$ | RI, MS      |
| 8  | β-Elemene                  | 1390 | 1389            | $2.2\pm0.2$                     | $\textbf{0.6} \pm \textbf{0.2}$ | $\textbf{0.6} \pm \textbf{0.1}$ | RI, MS      |
| 9  | Methyl eugenol             | 1400 | 1403            | $\textbf{0.1} \pm \textbf{0.1}$ | _                               | _                               | RI, MS      |
| 10 | Longifolene                | 1405 | 1407            | _                               | $\textbf{0.3} \pm \textbf{0.1}$ | _                               | RI, MS      |
| 11 | α-Gurjunene                | 1410 | 1409            | _                               | $0.6 \pm 0.2$                   | $1.3 \pm 0.2$                   | RI, MS      |
| 12 | β-Caryophyllene            | 1415 | 1417            | $\textbf{1.7} \pm \textbf{0.2}$ | $25.4 \pm 0.3$                  | $4.0 \pm 0.2$                   | RI, MS, Std |
| 13 | β-Copaene                  | 1430 | 1430            | $\textbf{0.1} \pm \textbf{0.1}$ | _                               | _                               | RI, MS      |
| 14 | γ-Gurjunene                | 1432 | 1431            | $0.3 \pm 0.2$                   | $1.5 \pm 0.2$                   | _                               | RI, MS      |
| 15 | Aromadendrene              | 1435 | 1439            | $0.5 \pm 0.2$                   | $1.6 \pm 0.1$                   | $5.6 \pm 0.2$                   | RI, MS      |
| 16 | α-Humulene                 | 1450 | 1452            | $0.9 \pm 0.2$                   | $2.6 \pm 0.2$                   | $0.9 \pm 0.1$                   | RI, MS      |
| 17 | Ishwarane                  | 1465 | 1465            | $4.3 \pm 0.3$                   | _                               |                                 | RI, MS      |
| 18 | Amorpha-4,7(11)-diene      | 1478 | 1479            | $4.6 \pm 0.2$                   | _                               | _                               | RI, MS      |
| 19 | α-Amorphene                | 1482 | 1483            | _                               | $1.4 \pm 0.2$                   | $4.9 \pm 0.2$                   | RI, MS      |
| 20 | Germacrene D               | 1484 | 1484            | $7.2 \pm 0.2$                   | $9.9 \pm 0.3$                   | $4.5 \pm 0.2$                   | RI, MS, Std |
| 21 | β-Selinene                 | 1485 | 1489            | _                               | _                               | $2.0 \pm 0.1$                   | RI, MS      |
| 22 | Eudesma-6,11-diene         | 1489 | 1489            | $0.9 \pm 0.2$                   | _                               |                                 | RI, MS      |
| 23 | cis-Eudesma-6,11-diene     | 1490 | 1489            | $0.9 \pm 0.2$                   | _                               | _                               | RI, MS      |
| 24 | (E)-Methyl isoeugenol      | 1490 | 1491            | $0.3 \pm 0.1$                   | _                               | _                               | RI, MS      |
| 25 | cis-Cadina-1,4-diene       | 1495 | 1495            | -                               | _                               | $1.3 \pm 0.2$                   | RI, MS      |
| 26 | Viridiflorene              | 1495 | 1496            | $0.7 \pm 0.2$                   | _                               | -                               | RI, MS      |
| 27 | α-Muurolene                | 1500 | 1500            | -                               | $1.3 \pm 0.1$                   | $2.3 \pm 0.2$                   | RI, MS      |
| 28 | Bicyclogermacrene          | 1501 | 1500            | _                               | $15.2 \pm 0.2$                  | $15.6 \pm 0.2$                  | RI, MS, Std |
| 29 | Epizonarene                | 1502 | 1501            | _                               | $0.4 \pm 0.2$                   | $1.0 \pm 0.1$                   | RI, MS      |
| 30 | ( <i>E,E</i> )-α-Farnesene | 1505 | 1505            | _                               | $1.0 \pm 0.1$                   | $1.3 \pm 0.1$                   | RI, MS      |
| 31 | Premnaspirodiene           | 1506 | 1505            | $0.4\pm0.1$                     | -                               | -                               | RI, MS      |
| 32 | γ-Cadinene                 | 1510 | 1513            | $5.5 \pm 0.3$                   | _                               | _                               | RI, MS      |
| 33 | 7-epi-α-Selinene           | 1520 | 1520            | J.J ± 0.J                       | $0.4 \pm 0.2$                   | $0.5 \pm 0.2$                   | RI, MS      |
| 34 | δ-Cadinene                 | 1522 | 1522            | 4.1 ± 0.2                       | $7.0 \pm 0.2$                   | $13.8 \pm 0.3$                  | RI, MS, Std |
| 35 | trans-Cadina-1,4-diene     | 1530 | 1532            | $0.1 \pm 0.2$                   | 7.0 ± 0.2                       | 15.8 ± 0.5                      | RI, MS      |
| 36 | $\alpha$ -Cadinene         | 1535 | 1537            | $0.1 \pm 0.1$<br>$0.5 \pm 0.2$  | 0.2 ± 0.1                       | 0.4 ± 0.2                       | RI, MS      |
| 37 | α-Cadmene<br>α-Calacorene  |      | 1544            | 0.5 ± 0.2                       | 0.2 ± 0.1                       |                                 | RI, MS      |
|    |                            | 1545 |                 |                                 |                                 | $0.2 \pm 0.1$                   | -           |
| 38 | Elemol                     | 1546 | 1548            | -                               | -                               | $0.5 \pm 0.1$                   | RI, MS      |
| 39 | Germacrene B               | 1560 | 1559            | $0.2 \pm 0.1$                   | $1.0 \pm 0.2$                   | $\textbf{0.6} \pm \textbf{0.1}$ | RI, MS      |
| 40 | β-Calacorene               | 1564 | 1564            | $0.1\pm0.1$                     | -                               | _                               | RI, MS      |
| 41 | Spathulenol                | 1575 | 1577            | -                               | $0.6 \pm 0.2$                   | $2.0\pm0.2$                     | RI, MS      |
| 42 | Globulol                   | 1590 | 1590            | _                               | 5.1 ± 0.3                       | -                               | RI, MS      |
| 43 | Viridiflorol               | 1592 | 1592            | _                               | $4.4 \pm 0.1$                   | $3.7 \pm 0.3$                   | RI, MS      |
| 44 | Junenol                    | 1610 | 1618            | -                               | $0.5 \pm 0.2$                   | _                               | RI, MS      |
| 45 | 1,10-di-epi-Cubenol        | 1615 | 1618            | $13.4 \pm 0.3$                  | _                               | _                               | RI, MS, Std |
| 46 | 1-epi-Cubenol              | 1625 | 1627            | -                               | $0.7 \pm 0.2$                   | _                               | RI, MS      |
| 47 | <i>epi-</i> α-Muurolol     | 1640 | 1640            | _                               | $2.7 \pm 0.2$                   | _                               | RI, MS      |
| 48 | Cubenol                    | 1645 | 1645            | _                               | _                               | $3.4 \pm 0.2$                   | RI, MS      |
| 49 | β-Eudesmol                 | 1646 | 1649            | _                               | $0.9 \pm 0.2$                   | $0.9\pm0.2$                     | RI, MS      |
| 50 | α-Cadinol                  | 1650 | 1652            | $40.7\pm0.2$                    | $3.8 \pm 0.2$                   | $4.1\pm0.2$                     | RI, MS, Std |
| 51 | Shyobunol                  | 1685 | 1688            | $\textbf{0.1} \pm \textbf{0.1}$ | _                               | -                               | RI, MS      |

Table 1: (continued)

| No                        | Components | ΚΙ² | ΚI <sup>b</sup> | Percentage (%) |      |      | Methods⁴ |
|---------------------------|------------|-----|-----------------|----------------|------|------|----------|
|                           |            |     |                 | CIPO           | CIFO | CRGO |          |
| Group components          |            |     |                 |                |      |      |          |
| Sesquiterpene hydrocarbon |            |     | 36.4            | 72.7           | 68.4 |      |          |
| Oxygenated sesquiterpenes |            |     | 54.2            | 18.7           | 14.6 |      |          |
| Phenylpropanoids          |            |     | 1.0             | _              | _    |      |          |
| Identified components (%) |            |     | 91.6            | 91.4           | 83.0 |      |          |

CIPO - C. impressa oil; CIFO - C. infectoria oil; CRGO - C. rugulosa oil.

phase, and genetic modifications. These factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic compounds [18].

Nevertheless, the abundant levels of  $\alpha$ -cadinol in the essential oil of C. impressa identified in this study represent the first chemical compositional report for this genus. Likewise,  $\alpha$ -cadinol has been identified as the major and most common constituent of sesquiterpenoids present in some species of the Lauraceae family such as Machilus mushaensis (Taiwan: leaf oil 20.8%) [19], Cinnamomum perrottetii (India: leaf oil 20.5%) [20], Beilschmiedia madang (Malaysia: leaf and bark oil 10.6%) [21], Neolitsea parvigemma (Taiwan: leaf oil 10.2%) [22], Lindera chunii (China: flowers, leaves and stems oil 8.6%) [23], and Litsea acutivena (Taiwan: twig oil 7.7%) [24]. α-Cadinol is an essential component found in European and Western Asian folk medicine and is used to treat inflammatory conditions, In addition to having a relatively good antifungal activity, it can be broadly applied as an antiseptic, anti-inflammatory, and cicatrising agent, while displaying some antibacterial and antiviral properties [25, 26].

## 4 Conclusion

The chemical composition of the essential oils of *C. impressa*, *C. infectoria*, and *C. rugulosa* plants growing in Malaysia were investigated for the first time using the GC and GC-MS method. These results shed further light into the phytochemistry of this unexplored species of the Flora of Malaysia. The next step is to evaluate the biological activities of the essential oil in order to valorise this species with a unique ecological character.

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 $<sup>^{</sup>a}$ Linear retention index, experimentally determined using homologous series of  $C_{6}-C_{30}$  alkanes.

bLinear retention index taken from Adams, Wiley, FFNSC2 or NIST08 and literature.

<sup>&#</sup>x27;Relative percentage values are means of three determinations ±SD

dIdentification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases; RI, based on comparison of calculated RI with those reported in Adams, FFNSC2 and NIST08.

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