



## Chemical Composition of Essential Oil of *Baeckea frutescens* L.

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### Authors' contributions

This work was carried out in collaboration between all authors. Author TDT designed the study and supervised author DND in the extraction of essential oil. Author DND collected the sample and performed the identification. Author TDT carried out the GC and GC/MS analyses and performed the statistical analysis. Both authors TOO and IAO managed the literature search and while author IAO wrote the first draft of the manuscript with assistance from author TOO. All authors read and approved the final manuscript.

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### ABSTRACT

The volatile components of the leaf oil of *Baeckea frutescens* L. (*Myrtaceae*) from the Hatinh Province, Vietnam, were analysed by capillary gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) and co-elution techniques. The identified compounds constituted more than 99.5% of the oil contents. Forty-nine compounds have been characterized

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among which  $\alpha$ -humulene (19.2%),  $\beta$ -caryophyllene (17.3%), baeckeol (13.8%),  $\alpha$ -thujene (8.8%), linalool (5.6%) and 1, 8-cineole (5.6%) were the major constituents. This result may represent another chemotype of the oil of *B. frutescens*.

**Aims:** Vietnam is a country blessed with many plants whose chemical compounds have not been previously examined. The aim of this research is to investigate the chemical constituents of essential oil of *Baeckea frutescens*.

**Study Design:** Extraction of essential oil from the air-dried leaf samples of *B. frutescens* and investigation of its chemical constituents.

**Place and Duration of Study:** Mature leaves of *B. frutescens* were collected from Hatinh Province, Vietnam in October 2013.

**Methodology:** Air-dried and pulverized leaves were subjected to hydrodistillation in accordance with Vietnamese Pharmacopoeia specification to obtain essential oil. The components of the oil were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) and co-elution techniques.

**Results:** Forty-nine compounds have been characterized among which  $\alpha$ -humulene (19.2%),  $\beta$ -caryophyllene (17.3%), baeckeol (13.8%),  $\alpha$ -thujene (8.8%), linalool (5.6%) and 1, 8-cineole (5.6%) were the major constituents.

**Conclusion:** The present oil compositions were found to be different from the results obtained previously from the essential oils of *B. frutescens* grown in other parts of the world. The present result may represent another chemotype of the oil of *B. frutescens*.

**Keywords:** *Baeckea frutescens*; essential oil composition;  $\alpha$ -humulene;  $\beta$ -caryophyllene; baeckeol.

## 1. INTRODUCTION

*Baeckea frutescens* L. is a shrub or small tree of the family *Myrtaceae*. The leaves are opposite, strip or strips tapered, 5-8 mm long, while the solitary flower is bisexual, yellow and white, about 2-3 mm in diameter. The fruits are small, about 1 mm while the seeds have horns. Flowering takes place between July and October [1]. Extracts from the leaves of *B. frutescens* have displayed anti-inflammatory and antioxidant [2] and cytotoxicity [3] activities. Flavonoids, chromones, sterols [4] and flavonol glycoside [5] were previously isolated from the plant. A biflavonoid compound, 3-O- $\alpha$ -l-rhamnopyranosylmyricetinyl-(1-2",11-2")-3-O- $\alpha$ -l-rhamnopyranosylmyricetin, present in *B. frutescens* was found to be useful in the prevention of arteriosclerosis [6]. The antioxidant and cytoprotective activities of the biflavonoid baeckein E characterized from the plant has been reported [7], while baeckein I displayed potent anti-inflammatory effect [8]. Other known compounds include baeckein A-D [9,10]. Two phloroglucinols with strong cytotoxicity activity against leukaemia cells (L 1210) were isolated from the dried leaves of *B. frutescens* [11]. 5-Hydroxy-2-isopropyl-7-methoxychromone isolated from the aerial parts of *B. frutescens* exhibited toxicity to the brine shrimp *Artemia salina* [12].

Literature information has shown that reports are available on compositions of essential oils from the leaf and twigs of *B. frutescens*. In a report, *p*-cymene (20.1%),  $\beta$ -caryophyllene (13.7%) and baeckeol (10.1%) were found as the main components of its leaf essential oil [13]. A high contents of 1,8-cineole (11.63%), linalool (9.58%),  $\delta$ -2-carene (7.76%), *p*-cymene (7.76%), caryophyllene (7.32%) and terpinen-4-ol (7.23%) were reported in the leaf and twig oils [14], while tasmanone, a non-terpenic triketone, was recently characterized in another sample [15]. Also, *B. frutescens* oil was reported [16] to consist mainly of  $\alpha$ -thujene (22.9%), 1,8-cineole (14.16%) and linalool (11.48%).

Previously  $\alpha$ -pinene and *trans*-carveol were described as major constituents of an undefined part of the plant from China [17] while the samples from Malaysia contained variations in their chemical compositions. One sample had an abundance of  $\alpha$ -pinene (18.2%),  $\beta$ -pinene (37.2%) and borneol (8.9%), another contained  $\alpha$ -pinene (20.9%),  $\beta$ -pinene (19.0%) and  $\gamma$ -terpinene (17.0%), the third sample contained  $\alpha$ -pinene (48.2%),  $\gamma$ -terpinene (19.7%) and linalool (8.6%) while  $\gamma$ -terpinene (34.1%),  $\alpha$ -humulene (10.6%), *p*-cymene (9.6%) were present in the fourth sample [18]. Another analysed leaf oil sample from Malaysia [19] was found to be made up of terpinolene (22.33%), *trans*- $\alpha$ -bisabolene 10.12% and *p*-cymene (9.85%). The essential

oil from an undefined plant part [20] had its major constituents as  $\beta$ -pinene (25.1%),  $\gamma$ -terpinene (12.3%), *p*-cymene (11.1%). The major components of *B. frutescens* [20] were  $\alpha$ -thujene (5.9%), limonene (11.1%) and 1,8-cineole (10.1%).

In this paper, the chemical compounds of essential oil obtained from *B. frutescens* are being reported, as part of an extensive research on the chemical analysis of poorly studied species of Vietnamese flora [21].

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection

Leaves of *B. frutescens* were collected in October 2013, in Nghi Xuan District (12.55 N, 109.07 E), Ha Tinh Province, Vietnam. Botanical identification of the plant was performed by Dr. D.N. Dai. The soil at the point of collection was saline and acidic in nature while the temperature was 10°C. A voucher specimen (DND 127) was deposited at the Botany Museum, Vinh University, Vietnam. 600 g of the plant was collected initially prior to air-drying. Plant samples were air-dried prior to extraction.

### 2.2 Extraction of the Volatile Oil

0.5 kg of air dried sample was shredded and their oils were obtained by hydrodistillation for 3 h at normal pressure, according to the Vietnamese Pharmacopoeia [22]. The yield of essential oil was 0.52% (v/w, light yellow), calculated on a dry weight basis.

### 2.3 Gas Chromatography (GC) Analysis of the Oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25  $\mu$ m, Agilent Technology). The analytical conditions were: carrier gas  $H_2$  (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 40°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0  $\mu$ L. Inlet pressure was 6.1 kPa. The sample was analyzed thrice.

### 2.4 Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25  $\mu$ m) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

### 2.5 Identification of the Constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST (Database 69) and Wiley 9<sup>th</sup> Version and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values [23,24].

## 3. RESULTS AND DISCUSSION

Of the 55 components of the essential oil of *B. frutescens* from Vietnam that were separated by GC, forty-nine compounds were identified after GC/MS analysis, representing 99.5% of the total oil (Table 1). Ubiquitous terpene compounds were the main classes of compounds identified in the oil, comprising of 19.5% monoterpene hydrocarbons, 16.4% oxygenated monoterpenes, 45.4% sesquiterpene hydrocarbons and 17.4% oxygenated sesquiterpenes. The major constituents were identified as  $\alpha$ -humulene (19.2%),  $\beta$ -caryophyllene (17.3%) and baeckeoal (13.8%). Other less predominant compounds were terpinen-4-ol (3.7%),  $\delta$ -cadinene (3.3),  $\gamma$ -terpinene (3.1%),  $\alpha$ -pinene (1.8%), *p*-cymene (1.6%),  $\alpha$ -terpineol (1.6%),  $\alpha$ -terpinene (1.2%),  $\alpha$ -terpinolene (1.1%),  $\beta$ -bourbonene (1.1%) and (*E*)-nerolidol (1.0%) while the rest had content lower than 1.0%.

The chemical constituents of essential oils of *B. frutescens* were previously studied from three countries namely China, Malaysia and Vietnam. From this result and the literature data, it appears

that *B. frutescens* essential oil exhibits high chemical variability. The major monoterpene constituents ( $\geq 10\%$ ) that were common to the essential oils includes  $\alpha$ -thujene,  $\alpha$ -pinene,  $\delta$ -2-carene,  $\beta$ -pinene, *p*-cymene, limonene, 1,8-cineole,  $\gamma$ -terpinene, terpinolene, linalool while the sesquiterpene compounds ( $\geq 10\%$ ) comprised of *trans*- $\alpha$ -bisabolene,  $\beta$ -caryophyllene,  $\alpha$ -humulene and baeckeol [13-20,25]. The compositional pattern of these monoterpenes and sesquiterpenes compounds however differed from one analysis to another. Moreover, the non-terpenic ketone, tasmanone ( $>10\%$ ) was previously described as a major compound of the oil [15]. Linalool and 1,8-cineole were also present in the essential oil of *B. frutescens* [26,27]. A comparison of different extraction methods revealed that solid-phase microextraction (SPME) gave oil whose major constituents were  $\gamma$ -terpinene, *o*-cymene,  $\alpha$ -pinene and 1,8-cineole. However, head-space extraction (HS), conventional hydrodistillation (HD) and microwave assisted hydrodistillation (MAHD) detected  $\gamma$ -terpinene,  $\alpha$ -pinene and *o*-cymene as major components [28].

When compared with previous studies, it could be seen that only  $\delta$ -2-carene and *trans*- $\alpha$ -bisabolene were the only compounds that could not be detected in the present study. However, the chemical combination of  $\alpha$ -humulene/ $\beta$ -

caryophyllene/baeckeol could be described as a new chemical composition of essential oil of *B. frutescens*. It may therefore represent a new chemotype of essential oil of *B. frutescens*. The chemical variations in the oil composition of *B. frutescens* by different researchers may be due some factors such as collection time, age of the plant, chemotypes, handling conditions, mode of extraction as well differences in geographical and climatic factors between these countries.

The essential oil of *B. Frutescens* was shown to possess anti-fungal activity against the mycelial growth of five pathogenic fungi [29]. The biological activities of an essential oil may be due to the major compounds or synergy between the major and some minor compounds present in the oil [30]. Referring to literature,  $\beta$ -caryophyllene is known to exhibit both antimicrobial and insecticidal activities [30]. Essential oils with moderate to high contents of  $\alpha$ -humulene and  $\beta$ -caryophyllene have been used to treat itching and other skin problems [31]. Baeckeol and its several analogues have demonstrated potent antioxidant, anti-inflammatory and cytotoxicity activities [7]. Therefore, considering the information on the biological potentials of the compounds present in therein, it may be postulated that the essential oil of *B. frutescens* may be useful as phytopharmaceuticals.

**Table 1. Percentage composition of the distilled aerial parts oil of *B. frutescens***

| Compounds <sup>a</sup> | RI (Cal.) | RI (Lit.) | Percent composition (% , $\pm$ SD) |
|------------------------|-----------|-----------|------------------------------------|
| $\alpha$ -Thujene      | 930       | 921       | 8.8                                |
| $\alpha$ -Pinene       | 939       | 932       | 1.8                                |
| $\alpha$ -Fenchene     | 956       | 945       | Tr                                 |
| Sabinene               | 976       | 969       | 0.4                                |
| $\beta$ -Pinene        | 980       | 974       | 0.3                                |
| $\beta$ -Myrcene       | 990       | 988       | 0.3                                |
| $\alpha$ -Phellandrene | 1006      | 1002      | 0.2                                |
| $\alpha$ -Terpinene    | 1017      | 1014      | 1.2                                |
| <i>p</i> -Cymene       | 1026      | 1020      | 1.6                                |
| Limonene               | 1032      | 1024      | 0.7                                |
| 1,8-Cineole            | 1034      | 1026      | 5.4                                |
| $\gamma$ -Terpinene    | 1061      | 1054      | 3.1                                |
| $\alpha$ -Terpinolene  | 1090      | 1086      | 1.1                                |
| Linalool               | 1100      | 1095      | 5.6                                |
| Terpinen-4-ol          | 1177      | 1174      | 3.7                                |
| <i>p</i> -Cymen-8-ol   | 1180      | 1179      | 0.1                                |
| $\alpha$ -Terpineol    | 1186      | 1186      | 1.6                                |
| $\alpha$ -Cubebene     | 1351      | 1345      | Tr                                 |
| $\alpha$ -Copaene      | 1377      | 1374      | 0.2                                |
| $\beta$ -Maaliene      | 1380      | 1380      | 0.7                                |
| $\beta$ -Bourbonene    | 1385      | 1387      | 1.1                                |

| Compounds <sup>a</sup>              | RI (Cal.) | RI (Lit.) | Percent composition (%; $\pm$ SD) |
|-------------------------------------|-----------|-----------|-----------------------------------|
| $\beta$ -Elemene                    | 1391      | 1389      | 0.1                               |
| $\alpha$ -Gurjunene                 | 1412      | 1409      | 0.2                               |
| $\beta$ -Caryophyllene              | 1419      | 1417      | 17.3                              |
| Calarene ( $\beta$ -Gurjunene)      | 1434      | 1431      | 0.3                               |
| Aromadendrene                       | 1441      | 1439      | 0.1                               |
| $\alpha$ -Humulene                  | 1454      | 1452      | 19.2                              |
| $\alpha$ -Elemene                   | 1477      | 1474      | 0.5                               |
| $\gamma$ -Muurolene                 | 1480      | 1478      | 0.2                               |
| $\alpha$ -Amorphene                 | 1485      | 1483      | 0.7                               |
| $\beta$ -Selinene                   | 1486      | 1486      | 0.3                               |
| $\alpha$ -Selinene                  | 1493      | 1498      | 0.3                               |
| Cadina-1,4-diene                    | 1496      | 1496      | 0.2                               |
| $\alpha$ -Muurolene                 | 1500      | 1500      | 0.4                               |
| ( <i>E,Z</i> )- $\alpha$ -Farnesene | 1508      | 1505      | Tr                                |
| $\delta$ -Cadinene                  | 1525      | 1522      | 3.3                               |
| $\alpha$ -Cadinene                  | 1538      | 1537      | 0.2                               |
| $\alpha$ -Calacorene                | 1546      | 1544      | 0.1                               |
| Elemol                              | 1550      | 1548      | 0.2                               |
| ( <i>E</i> )-Nerolidol              | 1563      | 1561      | 1.0                               |
| Caryophyllene oxide                 | 1583      | 1581      | 0.6                               |
| Humulene-6,7-epoxide                | 1593      | 1600      | 0.2                               |
| $\gamma$ -Eudesmol                  | 1629      | 1630      | Tr                                |
| $\beta$ -Eudesmol                   | 1651      | 1649      | 0.6                               |
| $\alpha$ -Eudesmol                  | 1652      | 1652      | Tr                                |
| $\alpha$ -Cadinol                   | 1654      | 1652      | 0.4                               |
| 7- <i>epi</i> - $\beta$ -Bisabolol  | 1661      | 1656      | 0.6                               |
| Tasmanone                           | 1720      | 1726      | 0.8                               |
| Baeckeol                            | 1861      | 1861      | 13.8                              |
| Total                               |           |           | 99.5                              |
| Monoterpene hydrocarbons            |           |           | 19.5                              |
| Oxygenated monoterpenes             |           |           | 16.4                              |
| Sesquiterpene hydrocarbons          |           |           | 45.4                              |
| Oxygenated sesquiterpenes           |           |           | 17.4                              |
| Others                              |           |           | 0.8                               |

<sup>a</sup> Elution order on HP-5MS column; RI (cal.), retention indices on HP-5MS column; RI (Lit.), literature retention indices; Tr, trace amount, <0.1%; SD, standard deviation, values were not significant for consideration and were omitted from the table to avoid congestion

#### 4. CONCLUSION

The chemical analysis of the essential oil of the leaf of *B. frutescens* led to the delineation of a new chemotype. It was also observed that significant variations could be seen in the chemical compositions of essential oils of this plant species. This chemical variation between different points of collection may ultimately be responsible for the variations in the biological potentials of essential oils of these from one country to another.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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