Identification of β-Caryophyllene (BCP) in Aceh patchouli essential oil (PEO) using gas chromatography-mass spectrophotometry (GC-MS)

E Sufriadi1,2,4, H Melina3,4, A A Munawar4,5, S Muhammad3,4 and R Idroes2,6,*

1 Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
2 Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
3 Chemical Engineering Department, Faculty of Engineering, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
4 Atsiri Research Center (ARC) PUI Nilam Aceh, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
5 Agricultural Technology Department, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
6 Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

*E-mail: rinaldi.idroes@unsyiah.ac.id

Abstract. This study aimed to determine the composition of β-Caryophyllene (BCP) content in patchouli oil from various regions in Aceh. The data from GC-MS analysis can also describe the type of BCP isomer most commonly found in Aceh patchouli. This research used Aceh Pogostemon cablin Benth patchouli oil from 17 different locations. Samples were analyzed using TSQ™ 9000 Triple Quadrupole GC-MS/MS (Thermo Scientific) with TraceGOLD TG-1MS GC Columns. The mean BCP retention time was 14.64 minutes, with a standard deviation (SD) of 0.16. Only one type of BCP compound isomer appeared, namely Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*, 4Z, 9S*)]. The results also showed that the average BCP level was 5.51%. Aceh patchouli contains a much higher BCP level than the minimum commercial target (3.4-3.5%). The MS spectrum data also showed a high similarity between the patchouli oil sample fragmentation and the compound library.

1. Introduction
Indonesia has great biodiversity provided by its vast forest [1]. It can produce endless resources of natural product which has benefit in the medicinal field [2], [3]. The natural products are easily found in the plant as a metabolite produced by itself [4]–[6] or by endophyte [7], [8]. The metabolite provides various medical activity to be antimicrobial [9], [10], antibacterial [11]–[13], antbiofilm [14], and antioxidant [15], [16].
BCP is one of the secondary metabolites found in many plants and essential oils such as copaiba, cannabis, rosemary, melissa, black pepper, cloves, and patchouli. BCP is classified into a bicyclic sesquiterpene compound. The sesquiterpene framework is made up of 15 carbon atoms and 24 hydrogen atoms. The compounds of the sesquiterpenes group generally have a straight, one-ring, or two-ring backbone like BCP. As BCP has these two rings, it is considered bicyclic. BCP is a sesquiterpene compound currently researched to determine its benefits in the medical and pharmaceutical fields. BCP has been extensively studied to treat several neurological diseases. More than 500 scientific publications have investigated this terpene's activity and found that it exhibits a protective role in several nervous system disorders, including pain, anxiety, seizures, depression, alcoholism, and Alzheimer's disease [17]–[19].

Among all BCP sources, patchouli is the great one that needs to be explored further. It is an endemic plant in Aceh and has been a livelihood source for the Acehnese farmers for more than a century [20]. The presence of BCP in each plant varies widely. In some plants, BCP is present as a major component, but it is minor in others.

Past research shows that BCP presence in patchouli is included in the minor category; however, it remains to be studied whether BCP benefits humans. Few studies specifically examine the pharmacological effects of patchouli BCP either in vitro or in vivo. However, before researching that, it is essential to study the real presence of BCP content in patchouli from various regions in Aceh.

IUPAC named the BCP compound \((1R, 4E, 9S)-4,11,11\text{-trimethyl-8-methylenebicycle}\[7.2.0\]undec-4-en\) with a molecular weight of 204 g/mol. Three types of BCP isomers are found in patchouli oil, namely (E)-β-caryophyllene, (E)-7-epi-β-caryophyllene, and (Z)-β-caryophyllene [21].

The purpose of this study was to determine the composition of BCP content in patchouli oil originating from various regions in Aceh. Analysis from the GC can also describe the type of BCP isomer most commonly found in Aceh patchouli.

2. Materials and methods
2.1. Materials and Samples.
This study used Aceh Pogostemon cablin Benth patchouli oil from 17 different locations. Seventeen patchouli oil samples were stored in glass bottles and then taken to the Laboratory of Chemical Instruments in the Faculty of Mathematics and Natural Sciences, Syiah Kuala University. Each sample is labeled N1 to N17 [22], [23].

2.2. Instrumentation and chromatographic conditions
Samples were analyzed using the TSQ™ 9000 Triple Quadrupole GC-MS/MS (Thermo Scientific) with TraceGOLD TG-1MS GC Columns, equipped with a CombiPAL Autosampler (Basel, Switzerland), consisting of a split/splitless injector port and a mass spectrometer detector. The injection is carried out in splitless mode. The GC system is equipped with a DB-5 column (30m × 0.25mm × 0.25mm). The carrier gas is helium (1.0 mL/min). The oven temperature is programmed from 60 °C for 3 minutes in increments of 40 °C/minute to 300 °C. The chromatography process was finalized in 9 minutes. The mass detector is operated by an impact electron system at 70 eV. The signal is recorded and processed by GC/MS Data Analysis Software [24].

2.3. Determination of BCP in the sample
In this study, BCP analysis was performed by inserting a 1.0 ml syringe into the GC port, which is equipped with a 10 mL clear glass bottle with an 18 mm magnetic screw cap (Thermo Scientific).

3. Results and discussion
The chemical composition results in Aceh patchouli oil found that the average β-Caryophyllene (BCP) retention time was 14.64 minutes with a standard deviation (SD) of 0.16. This SD value shows that there is consistency in separating BCP analytes from various sources and locations of patchouli oil in Aceh.
This data is also supported by the type of BCP compound isomer that appears in only one type, namely Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)] as shown in Table 1 [25].

Table 1. Retention time, the composition of BCP content in each patchouli oil sample, and the appearing compound.

| Sample Code | Retention Time (min) | BCP Content (%) | Library Compound |
|-------------|----------------------|-----------------|------------------|
| N1          | 14.62                | 4.41            | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)] |
| N2          | 14.63                | 4.68            |                  |
| N3          | 14.61                | 4.74            |                  |
| N4          | 14.63                | 4.94            |                  |
| N5          | 14.41                | 4.56            |                  |
| N6          | 14.58                | 3.20            |                  |
| N7          | 14.54                | 3.17            |                  |
| N8          | 14.17                | 2.18            |                  |
| N9          | 14.77                | 8.59            |                  |
| N10         | 14.76                | 7.79            |                  |
| N11         | 14.73                | 5.92            |                  |
| N12         | 14.77                | 5.90            |                  |
| N13         | 14.76                | 7.00            |                  |
| N14         | 14.74                | 8.50            |                  |
| N15         | 14.70                | 6.87            |                  |
| N16         | 14.69                | 7.02            |                  |
| N17         | 14.79                | 4.20            |                  |
| Avg         | 14.64                | 5.51            |                  |
| SD          | 0.16                 | 1.89            |                  |

Remark: N1 = Gunung Pudung, South Aceh; N2 = Pucok Krueng, South Aceh; N3= Alur Mas, South Aceh; N4 = Paya Dapur, South Aceh; N5 = Kluet Selatan, South Aceh; N6 = Menggamat, South Aceh; N7 = Lawe Sawah, South Aceh; N8 = Meukek South Aceh; N9 = Panga Aceh Jaya; N10 = Nisam Aceh Utara; N11 = Sungai Mas, Aceh Barat; N12 = Woyla, Aceh Barat; N13 = Sampoiniet, Aceh Jaya; N14 = Keumala, Pidie; N15 = Subulussalam Town; N16 = Subulussalam (drum); and N17 = Great Aceh.

The results also showed that the average BCP level was 5.51%. This value is commercially potential. The minimum target for patchouli oil is only around 3.4-3.5%, but patchouli oil from Aceh is much higher. The BCP content also found that the SD value reached 1.89, meaning that the distribution of BCP content between locations in Aceh was extensive, starting from 2.18% as the lowest to 8.59% as the highest [26].

The MS spectrum data also shows a high similarity between the patchouli oil sample fragmentation and the compound library. Even though the percentage of similarities is very high, the MS data is only a comparison with the library data. However, during injection, the internal standard is used for the target compound we are looking for, making the results more valid than relying solely on library data (Figure-1) [27, 28].
Figure 1. The MS fragmentation pattern of the N1 patchouli oil sample was compared with the compound fragmentation predicted by the compound library.

4. Conclusion
BCP found in various Aceh patchouli oils tended to contain the same isomer, namely Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R- (1R *, 4Z, 9S *)]. Chemotaxonomy shows the similarity in patchouli's origin, but this assumption still needs to be validated by using reference material. Potentially, BCP's presence in Aceh patchouli oil is very potential because the average BCP content is 5.51%.

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