Investigation of Production Process and GC-MS Analysis of Chemical Constituents of Three Traditional Medicines Sumbawa Oils

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ABSTRACT

The aims of this research are to study the production process and the chemical composition of three traditional Sumbawa oils from Benete Village (BT), Batu Dulang Village (BD), and Hutan Sumbawa (HS). A survey was conducted to learn the production process; and the chemical composition of the Sumbawa oils was determined by fractionation method using three solvents with different polarity (methanol, dichloromethane (DCM) and n-hexane), followed by GC-MS analysis of each extract. In general, the three Sumbawa Oils were traditionally produced by mixing various species of medical plants into the coconut oil through heating process. Based on GC-MS analysis, the constituents of the Sumbawa oils are mainly fatty acid, fatty acid ester, monoterpene, and sesquiterpene. Three major identical compounds were identified in all three products with different percentages of abundance namely octadecanoic acid/stearic acid, hexadecanoic acid/palmitic acid, and methyl palmitate. Furthermore, there were other major compounds such as linoleic acid, lycopersene, and dodecanoic acid/lauric acid. The non-natural product compounds found in Sumbawa oil extracts and the biological activities of major compounds are discussed in this article.

Key word: Sumbawa oil, traditional medicine, GC-MS analysis

INTRODUCTION

The utilization of traditional medicine produced from natural products has recently become more attractive and popular in our society concerning for to the effectiveness to treat a variety of diseases. In developing countries, most populations depend on traditional medicine for their health care. It could be due to the perception that traditional medicine does not possess harmful side effects and the medication cost is less expensive compared to most modern drugs [1].

Sumbawa oil is one of the Indonesian traditional medicines produced in Sumbawa Island by mixing coconut oil and several medicinal plants via heating process. Sumbawaneses use Sumbawa oil as antispasmodic, antipyretic, and appetite-enhancing drug. In addition, it is well known to cure diseases such as diarrhea, snakebite, wound, bruise, twist, flaky skin, toothache, stomachache, indigestion, back pain, and skin diseases, to reduce the symptoms of arthritis, and to accelerate recovery postpartum maternal condition [2].
The Sumbawa oils existing in the society are mostly not standardized yet, and the analysis of chemical constituents in Sumbawa oil has not been conducted much. Accordingly, it is essential to address some considerations into its production processes and chemical compositions. The results of chemical analysis will then lead us to select the effective raw materials and to determine the production process based on the active compounds contained. Thus, the efficiency of the raw materials and the production processes can be improved.

To establish a more representative Sumbawa oil profile, three different products were evaluated. Although the methods primarily used in the production of Sumbawa oils are the same, the raw materials used are different. Therefore, the varied raw material selection is likely to cause differences in the metabolites contained in Sumbawa oil products.

**EXPERIMENT**

**Chemicals and instrumentation**

Sumbawa oils used in this study were three different products from Benete Village (West Sumbawa, Indonesia), Batu Dulang Village (Sumbawa, Indonesia), and Hutan Sumbawa (Sumbawa, Indonesia). The solvents used for fractionation were methanol p.a (Merck), dichloromethane (DCM) p.a (Merck) and n-hexane p.a. (Merck).

This study used GC-MS Shimadzu QP2010 ULTRA to determine the compound contained in every fraction of Sumbawa oils from three different products. The injection temperature was at 290°C. The initial column temperature was started at 40°C for 5 minutes and programmed to increase 80°C/min by heating until 280°C for 2 minutes. Meanwhile, MS was conditioned at temperature of transfer line at 260°C and ion source at 200°C. Ions were obtained by electron ionization mode. The RTX-5MS capillary column was used with length 30 m, diameter 0.25 mm and film thickness 0.25 μm. Helium was used as carrier gas with flow rate of 30 mL min⁻¹. Molecular mass range of ions was identified at 35-500 m/z.

**Procedure**

A survey was conducted to note the process of selection and preparation of plant materials, as well as steps of the production. For the determination of chemical constituents, liquid-liquid extraction and GC-MS analysis were implemented. Initially, the oil sample (100 mL) was extracted with methanol (3 x 100 mL) with occasional swirling to get methanol fraction and residue. The residue was then partitioned by using dichloromethane (DCM) (3 x 100 mL) to produce DCM fraction. The residue of DCM partition was extracted by n-hexane (3 x 100 mL) to get n-hexane fraction. The solvents of three fractions were removed under pressure by using rotary evaporator. After evaporation, three produced fractions were finally run by GC-MS to obtain the chemical identification and composition abundance. The abundances of chemical constituent were expressed as a percentage of total chemical abundance in the number of solvents found.

**RESULT AND DISCUSSION**

**The production process of Sumbawa oils**

Observation concerning the production of Sumbawa oils has been done by visiting and interviewing the oil producers. The survey of the Sumbawa Oils production in three locations, namely Benete Village (BT), Batu Dulang Village (BD), and Hutan Sumbawa (HS), revealed that Sumbawa Oil is traditionally produced in liquid form and made from coconut oil mixed with various kinds of plant roots, woods, and other materials that are usually dried before the mixing process.
The process started by producing half-cooked coconut oil using traditional wood-burning stove. The half-cooked coconut oil is then added to other medicinal plants, where these medicinal plants are previously mashed to enlarge the surface area. The mixture is then cooked with occasional stirring for 15 minutes. The next step is filtering and praying the oil by the oil producer (in Sumbawa language called Sandro) which is believed to increase the efficacy of Sumbawa oil. Finally, the Sumbawa oils are ready to be packaged and distributed to the market. In general, the production process of the three Sumbawa Oils is quite similar, except for the number and types of plant species used.

Chemical contents of Sumbawa oils

Several compounds found from the chemical analysis of Sumbawa Oils from three products using GC-MS can be seen in Table 1 below.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Abundance (%)</th>
<th>Similarity Index (SI)</th>
<th>Group of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>BD</td>
<td>HS</td>
</tr>
<tr>
<td>Octadecanoic acid/stearic acid</td>
<td>3.58</td>
<td>8.05</td>
<td>5.45</td>
</tr>
<tr>
<td>Hexadecanoic acid/palmmitic acid</td>
<td>9.37</td>
<td>5.79</td>
<td>14.32</td>
</tr>
<tr>
<td>Methyl hexadecanoate/methyl palmitate</td>
<td>2.48</td>
<td>11.24</td>
<td>1.82</td>
</tr>
<tr>
<td>Delta decalactone</td>
<td>0.42</td>
<td>1.58</td>
<td>-</td>
</tr>
<tr>
<td>Tetradecanoic acid/myristic acid</td>
<td>2.98</td>
<td>16.28</td>
<td>-</td>
</tr>
<tr>
<td>Dodecanoic acid/lauric acid</td>
<td>3.41</td>
<td>27.07</td>
<td>-</td>
</tr>
<tr>
<td>N-pentanal</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,1,2-trichloroethane</td>
<td>1.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl octanoate/methyl caprylate</td>
<td>1.91</td>
<td>-</td>
<td>1.98</td>
</tr>
<tr>
<td>N-tridec-1-ene</td>
<td>0.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trans-2-tridecenal</td>
<td>0.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E,E)-2,4-decadienal</td>
<td>4.39</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl decanoate/methyl caprate</td>
<td>1.57</td>
<td>11.58</td>
<td>-</td>
</tr>
<tr>
<td>Methyl dodecanoate/methyl laurate</td>
<td>5.73</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl tetradecanoate/methyl myristate</td>
<td>3.36</td>
<td>23.51</td>
<td>-</td>
</tr>
<tr>
<td>Cis-9-Octadecen-1-ol</td>
<td>0.86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heptadecene-(8)-carbonic acid-(1)</td>
<td>0.81</td>
<td>6.73</td>
<td>-</td>
</tr>
<tr>
<td>Hexadecanoic acid, 2-hydroxy-1,3-</td>
<td>0.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>propanediyl ester</td>
<td>(R)-(1)-14-methyl-8-hexadecyn-1-ol</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>2-monopalmitin</td>
<td>1.44</td>
<td>-</td>
<td>5.74</td>
</tr>
<tr>
<td>1-monolinolein</td>
<td>4.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Octanic acid/caprylic acid</td>
<td>0.54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-octadecanethiol</td>
<td>0.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl octadec-9-enolate/methyl oleate</td>
<td>2.71</td>
<td>-</td>
<td>2.69</td>
</tr>
<tr>
<td>Methyl octadecanoate/methyl stearate</td>
<td>1.62</td>
<td>1.88</td>
<td>-</td>
</tr>
<tr>
<td>Dodecanoic acid, 1-(hydroxymethyl)-1,2-</td>
<td>6.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ethanediyl ester</td>
<td>(R,2)-9,12-Octadecienoyl chloride</td>
<td>3.05</td>
<td>-</td>
</tr>
<tr>
<td>(Z,Z)-9,12-Octadecadienoic acid</td>
<td>21.07</td>
<td>-</td>
<td>44.70</td>
</tr>
<tr>
<td>acid/linoic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decanoic acid/capric acid</td>
<td>0.56</td>
<td>3.53</td>
<td>-</td>
</tr>
<tr>
<td>Octanoic acid/caprylyc acid</td>
<td>0.54</td>
<td>9.26</td>
<td>-</td>
</tr>
<tr>
<td>Dodecanoic acid, 2,3-dihydroxypropyl</td>
<td>-</td>
<td>1.43</td>
<td>-</td>
</tr>
</tbody>
</table>
NB. BT: Benete Village (West Sumbawa, Indonesia), BD: Batu Dulang Village (Sumbawa, Indonesia), and HS: Hutan Sumbawa (Sumbawa, Indonesia). Values in bold indicate compounds with the highest abundance found in some samples.

Based on Table 1, extracts of Sumbawa oils from Benete Village (BT), Batu Dulang (BD), and Hutan Sumbawa (HS) present several similarities and differences in their chemical constituents. There were 46 compounds identified by using GC-MS from all fractions, in which were 31 compounds from BT, 16 compounds from BD and 18 compounds from HS. Octadecanoic acid/stearic acid, hexadecanoic acid/palmitic acid, and methyl palmitate were compounds found in three products of Sumbawa Oil with different percent abundance in each product. Octadecanoic acid/stearic acid was found in Sumbawa oil products from BT, BD and HS with percentages of abundance 3.58, 8.05, and 5.45 respectively. Hexadecanoic acid/palmitic acid was also identified in all three products of Sumbawa oil and the percentages of abundance were 9.37% (BT), 5.79% (BD), and 14.32% (HS). Methyl palmitate is another identical compound found in Sumbawa oil extracts with the highest percentages of abundance found in BD (11.24%). In addition, there were three other major compounds identified in Sumbawa oils, namely linoleic acid, dodecanoic acid/lauric acid and lycopersene. Linoleic acid was detected as the most abundant compound with the percentages of abundance 21.07% (BT) and 44.70% (HS), followed by lycopersene (31.81% in HS) and dodecanoic acid/lauric acid, with the highest content observed in BD (27.07%).

The results of GC-MS analysis showed that the Sumbawa oils (BT, BD, and HS) are composed mainly of saturated fatty acids, unsaturated fatty acids, fatty acid esters, monoterpenes, and sesquiterpenes. Specifically, for Sumbawa oil HS, it is rich in monoterpenoid and sesquiterpene such as delta decalactone, 9-octadecanoic acid methyl ester, 1,8-cineole, trans-caryophyllene, zingiberene, beta-bisabolene, beta-sesquiphellandrene. Based on the literature, monoterpenes and sesquiterpenes have been found to show pharmacological potential as antimicrobial, anti-tumor, anti-inflammatory, antioxidant, anticancer, anti-fungal, and antipyretic [2], [3]. This indicates that the Sumbawa oil HS is likely to have better efficacy as traditional medicine than two other products (BD and BT).

The difference in chemical composition is caused by different spices and plant species used in BT, BD, and HS. Furthermore, even in the same plant species, the differences may be due to various factors such as geographic position, environmental and agronomic conditions, also due to the genetic variability of the plants [4], [5].
The effect of heating process in chemical constituents of Sumbawa oils

Sumbawa oil is traditionally produced by heating the mixture of coconut oil and medicinal plants, followed by stirring and filtering. The high temperature used in the production process could cause compositional changes in the oil via a complex series of chemical reactions including triacylglycerol (TAG) hydrolysis, oxidation, and polymerization [6].

In this study, non-natural product compounds formed under prolonged thermal process are assessed, such as (E,E)-2,4-decadienal and a group of fatty acid esters. (E,E)-2,4-decadienal is a lipid peroxidation product of linoleic acid [7]. Hexadecanoic acid, 2-hydroxy-1-3-propanediy1 ester is a fatty acid ester obtained by the formal acylation of positions 1 and 3 of glycerol by palmitic acid [8], while other fatty acid esters such as 1-monolinolein; methyl oleate; methyl stearate; octadecanoic acid, methyl ester; decanoid acid, methyl ester; tetradecanoic acid, methyl ester; and methyl palmitate are formed by esterification of fatty acids [9]. Apart from the changes in chemical composition during the thermal treatment, Sumbawa oil bioactive properties might be lost during the process, especially those susceptible to heat. This reveals that the heating process affects the structure of the chemicals in Sumbawa oil. Thus, to minimize adverse changes in the chemical composition of Sumbawa oil, treatment in lower temperature needs to be explored.

Bioactivities of major compounds in Sumbawa oils

GC-MS analysis successfully identified six major compounds from three Sumbawa oil products, with three of those compounds are identical compounds (i.e. octadecanoid acid/stearic acid, hexadecanoic acid/palmitic acid, and methyl hexadecanoate). Based on references, the following are the bioactivities of those compounds.

**Octadecanoic acid (stearic acid)**

Stearic acid (CH$_3$(CH$_2$)$_{16}$COOH) is a saturated fatty acid that is easily obtained from pork fat (lard), animal fats, cocoa, cocoa butter, butter, margarine, vegetable oils, fish, nuts, and seeds. Stearic acid is known to have potential as antibacterial, antifungal agent, and neuroprotective effect by protecting cortical neurons against oxidative stress by boosting the internal antioxidant enzymes. Stearic acid also has various roles in the metabolism of our body, including the production of several types of fat molecules needed to build cell membranes and for a variety of biochemical processes. Stearic acid is also used to form the stable creams, lotions and ointments in the production of deodorant, antiperspirant, cream base, and hand lotion [10], [11].

**Hexadecanoic acid (palmitic acid)**

Hexadecanoic acid or palmitic acid (CH$_3$(CH$_2$)$_{14}$COOH) is a saturated fatty acid found in palm oil, cocoa butter oil, cheese, butter, nuts, coconut, seed oil, peanut oil, olive oil, sunflower oil, and margarine. Hexadecanoic acid has an important role as antioxidant, hypcholesterolemic, nematicide, pesticide, lubricant, antianrogenic, flavor, hemolytic, anti-inflammatory, and antibacterial. Hexadecanoic acid has shown cytotoxicity to human leukemic cells, MOLT-4. It also shows in vivo antitumor activity in mice [12], [13], [14].

**Methyl hexadecanoic acid/methyl palmitate**

Methyl palmitate is a fatty acid ester (CH$_3$(CH$_2$)$_{14}$COOCH$_3$) which is a carboxylic ester usually obtained from vegetable oils and edible fats. Based on literature, methyl palmitate is proven to possess aracidal activity. Methyl palmitate has also been reported to inhibit
phagocytic activity and nitric oxide production of certain cells, reduce levels of tumor necrosis factor-alpha (TNFα), prostaglandin E2 (PGE2), and interleukin-10 (IL-10) without affecting ATP levels. Furthermore, this compound is widely used indirectly in a wide range of food, pharmaceutical, cosmetic and industrial applications [15], [16], [17], [18].

**Linoleic acid**
Linoleic acid is a double unsaturated fatty acid, also known as an omega-6 fatty acid, occurring widely in plant glycosides. Linoleic acid is a carboxylic acid with an 18-carbon chain and two cis double bonds; with the first double bond located at the sixth carbon from the methyl end. Linoleic acid plays important role in transport and metabolism of fat, immune function, maintain the function and integrity of cell membranes. Furthermore, linoleic acid is also known for its antibacterial, antidiabetic and antimalarial properties [19], [20].

**Dodecanoic acid (lauric acid)**
Dodecanoic acid or lauric acid (CH₃(CH₂)₁₀COOH) is saturated fatty acid composed of 12 carbon atoms, which is solid white at room temperature and easily melt when heated. Detailed studies show that lauric acid accounts for many of the properties of coconut oil. Among the saturated fatty acids, lauric acid has been shown to be very active against gram-positive bacteria and a number of viruses and fungi [4]. Additionally, lauric acid found in breast milk helps babies to fight against infections and strengthen the immune system. Furthermore, the lauric acid can inhibit tumor proliferation, protecting the intestines from pathogens overgrowth, and increasing the HDL (good cholesterol) in the blood [21], [22], [23], [24], [25], [26].

**Lycopersene**
Lycopersene is an organic compound (C₄₀H₆₆) found in leaf of Capparis diversifolia and Trichiliaconnaroides, and carrots. Lycopersene have been isolated and identified as intermediates in plant carotenogenesis. This compound is converted by a cell-free system from geranylgeranyl pyrophosphate, obtained from tomato fruit plastids. In addition, lycopersene has been identified as a compound with pharmaceutical potential as antimicrobial agent [27], [28], [29].

Based on the bioactivities of the major compounds in the product, Sumbawa oil has potency for other applications besides the traditional applications as antispasmodic, antipyretic, appetite-enhancing drug, antifungal, and antimicrobial. Some possible uses are as hypocholesterolemic, anti-inflammatory, and antidiabetic.

**CONCLUSION**
GC-MS analysis showed that there were six major compounds identified in Sumbawa oil products with different percentages of abundance. Three of those major compounds were identical compounds found in BT, BD and HS, namely hexadecanoic acid, octadecanoic acid and methyl palmitate. Other major compounds with known biological activities were linoleic acid (BT, HS), lycopersene (HS), and dodecanoic acid (BT, BD). The major constituents belong to fatty acid, fatty acid ester, and alkene groups.

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REFERENCES


