Chemical compounds in essential oil of nutmeg leaves
(Myristica fragrans) from Batang Indonesia

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Abstract. Nutmeg oil has an important function as natural tropical biomedicine activity which commonly found in Batang region. This essential oil is one of the dominant traditional export product from Batang instead of patchouli oil, Clove oil, and lemongrass oil. The objective of the research was to analyze chemical compound of essential oil of nutmeg leaves from Batang region produced by traditional destillation. Research methods was conducted by GCMS method. The biomedical activity against some tropical diseases was analysed in-silico. The results showed that essential oil from nutmeg leaves showed potential antimicrobial activities in silico against several tropical diseases in skin. The GCMS result showed 2-BETA.-PINENE and .gamma.-Terpinene as the major compound with values 34,46% and 30,28%.

1. Introduction
Nutmeg (Myristica fragrans) is commonly used in traditional medicine to treat rheumatism, relieve pain, malaria, and others. Some researchers have also found that nutmeg also has antimicrobial, insecticidal, antioxidant, anticancer, antidepressant, hepatoprotective, and many other biological activities[1-6]. Nutmeg essential oil had chemical compound consist of terpenes, monoterpen hydrocarbons, phenols, sesquiterpene hydrocarbons, monoterpen alcohol, esters, aromatics, various organic acid, and other compounds [7-9]. Cultivation of nutmeg is quite easy and this plant can live in marginal lands. The nutmeg plant is one of Indonesia's native plants. Nutmeg is included in the Myristicaceae family which consists of 15 genera and 250 species. The four clans of nutmeg are found in tropical regions of Asia [10]. Nutmeg has a high prospect and economic value as an essential oil commodity. Indonesia is the world's leading producer and exporter of nutmeg, mace, and nutmeg oil, which has a market share of 26.71 percent of the world market. The demand for nutmeg oil is quite high which always increases 3 - 5% each year[1]. The dominant nutmeg producing areas in Indonesia in 2018 were Maluku with a production of 13.008 tons, Aceh 6.754 tons, Sulawesi 6.603 tons, Papua 4.823 tons, Java 1.606 tons and Sumatra 1.579 tons[6]. Apart from these regions, nutmeg is also
predominantly produced from Java especially West Java [1,2,8,9]. Batang Regency is one of the nutmeg producing areas from Java Island, instead of Citronellal oil, Patchouli oil and Clove Oil [12-13]. The seeds, mace, fruit, bark and root as a part of nutmeg plants were contained secondary terpenoid metabolite compounds as aromatic and edible plant which have been traditionally used to treat various diseases [14,15]. The main constituents of essential oil from the different parts of *M. fragrans* were tend to similar which most of the differences found were quantitative including leaves. [15]. Unfortunately, the information about the quality and chemical composition of essential oil from nutmeg leaves, especially from Batang Regency was very limited. The aim of this study was to analyses the quality of the essential oil from nutmeg leaf from the Batang area by using the GCMS method and their antimicobial activity in silico.

2. Methods

2.1. Study area
Nutmeg leaves were collected from Blado District area. The essential oil is produced using distillation process of nutmeg leaves. The leaf were dried for 24 hours before distillation to easies and accelerates essential oil expenditure.

2.2. Nutmeg oil extraction
The extraction method was carried out using dry nutmeg leaves. Nutmeg oil distillation is performed by steaming the nutmeg leaves. The kettle is made of stainless steel. Steam consisting of a mixture of essential oil and water will come out of the kettle, then flow through a pipe connected to the condenser as coolant. The steam which contains a mixture of water and oil will drip down the pipe and be collected in a container followed by the separation process to obtain pure nutmeg oil.

2.3. GC-MS analysis
The gas chromatography-mass spectroscopy (GC-MS) method was used to determine the various chemical compounds contained in nutmeg leaf oil. The process of making nutmeg oil begins with separation using a capillary column measuring 30 x 0.25 mm (l x i.d.) coated with a 0.25 mm 5% phenylmethyl siloxane film. The reaction was carried out at a column temperature of 80 °C for injection purposes and mounted on a flame ionization detector (FID). The programming for the analytical reaction was set at 10 °C min-1 to 150 °C. The reaction is continued from 5 °C minute-1 to 250 °C and ends from 10 °C minute-1 to 280 °C and lasts for 5 minutes. Helium gas in this reaction is used as a carrier gas with a flow rate of 1 mL / minute using splitless injection, (2μL). The spectrometer used in this process is operated using the electron impact mode (EI), with an electron energy of 70 eV and a scanning range of 50-550 amu. High temperatures are used for inlet and ionization sources, namely 240 °C and 280 °C. The identification process of each component of nutmeg oil is carried out by comparing the retention time with the standard substances. This is also done by matching the mass spectral data with the MS and reference libraries (NIST and Wiley 275.l). The percentage area obtained by FID was used as the basis for quantitative analysis [12].

2.4. Determination of physico-chemical substances in nutmeg leaf oil
Determination of physicochemical substances and their structure in nutmeg leaf oil was carried out by chemical compound analysis using the Pubchem and Pubchem Bioassay facilities available at GenBank[16]
3. Result and discussion

Batang is one of the areas in Indonesia which has a lot of nutmeg plants. Many residents in this area traditionally process nutmeg into essential oils as exhibited in Figure 1. Nutmeg oil from the Batang region is clear yellowish white with a nutmeg aroma. Usually obtained through a steam distillation, water distillation or a combination of both. Nutmeg oil is sensitive to light and temperature but is insoluble in water [2]. The quality of the essential oil from nutmeg leaves from Batang region by GC-MS analysis was presented in Table 1.

![Figure 1. Myristica fragrans from Batang region (left) and Nutmeg oil (right)](image)

**Table 1. Chemical compound of essential oil of nutmeg leaves from Batang region Indonesia**

| No | RTime | Area % | Chemical Substances                                      |
|----|-------|--------|----------------------------------------------------------|
| 1  | 7.370 | 0.67   | \(\alpha\)-Thujene                                       |
| 2  | 7.708 | 30.28  | \(\gamma\)-Terpinene                                     |
| 3  | 7.971 | 0.48   | Camphene (CAS)                                           |
| 4  | 9.060 | 34.46  | 2-\(BETA\)-PINENE                                       |
| 5  | 9.584 | 3.20   | \(\beta\)-Myrcene                                        |
| 6  | 9.903 | 1.50   | 1-Phellandrene                                           |
| 7  | 10.180| 3.15   | \(\delta\)-3-Carene                                      |
| 8  | 10.343| 1.45   | (+)-2-CARENE                                            |
| 9  | 10.690| 2.73   | \(\beta\)-Phellandrene                                   |
| 10 | 10.806| 5.19   | dl-Limonene                                              |
| 11 | 11.745| 1.97   | \(\gamma\)-Terpinene                                     |
| 12 | 12.713| 2.64   | \(\alpha\)-TERPINOLENE                                   |
| 13 | 13.031| 1.39   | LINALOOL L                                              |
| 14 | 15.320| 2.73   | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS)     |
| 15 | 15.658| 0.55   | 3-Cyclohexene-1-methanol, \(\alpha\),\(\alpha\),4-trimethyl-, (S)- (CAS) |
| 16 | 18.296| 0.77   | 1,3-Benzodioxole, 5-(2-propenyl)- (CAS)                  |
| 17 | 20.187| 0.51   | Phenol, 2-methoxy-3-(2-propenyl)- (CAS)                  |
| 18 | 21.281| 0.54   | \(\alpha\)-Copaene                                       |
| 19 | 22.308| 0.52   | trans-Caryophyllene                                      |
| 20 | 24.329| 5.26   | CROWEACIN/Myristicin                                     |

100,00
The chemical composition of leaves nutmeg oil analysis by GCMS method gained 20 compounds. The main component of nutmeg oil from leaf are 2.-beta.-pinene (34,46%) and .gamma.-Terpinene (30,28%), as shown on Table 1. Both of this substances was revealed high peak in the chromatogram as illustrated in Figure 2. The result was supported by previous research which found that the chemical compound of leaf, mace, fruit pericarp and nutmeg were α-pinene, β-pinene, terpinen-4-ol, safrol, eugenol, isoeugenol, methyl isoeugenol, myristicin, and elemicin [15]. The similarity of the results of the two studies on the essential oil from leaf nutmeg is the presence of two main components and characteristics of the nutmeg essential oil, namely beta pinene and myristicin. The other researcher showed that beta-pinene and myristicin also found in seed instead of α-phellandrene, 3-carene, p-cymene, limonene, α-thujene, α-pinene, camphene, sabinene, myrcene, α-terpinene, γ-terpinene, and terpinolene [17]. The other researcher exhibited that in nutmeg essential oil of M. fragrans seed had the main component of monoterpen hidrokarbon like alpha pinene, beta pinene, sabinene 61-88%, monoterpenes acid 5-15% and aromatic ether like myristicin, elemycin and safrole about 2-18% [10].

**Figure 2.** Chromatogram of Nutmeg oil

Identification and analysis of predominant chemical compound from essential oil of M. fragrans by GCMS analysis based on data from NCBI (www.ncbi.nlm.nih.gov/pubchem):

1. 2.-BETA.-PINENE

Beta-Pinene has another name terbenthene or 2(10)-pinene. Beta-Pinene is a bicyclic monoterpenoid organic compound containing exactly 2 rings attached to one another. Beta-Pinene is a solid and is water insoluble and relatively neutral. Beta-pinene is found in cell membranes and cytoplasm. Beta-Pinene has a green color. 2.-Beta.- Pinene as illustrated in Fig. 3 is a pinene isomer having an exocyclic double bond. 2.-Beta.- Pinene is an essential oil component of many plants because it is a plant metabolite[12]. β-Pinene was one of the main component from nutmeg oil that responsible for antioxidant, and antimicrobial activity like *Staphylococcus aureus, S. epidermis, Shigella disenteriae* and *Salmonella typhi*[9,17,19].

**Figure 3.** Chemical structure of 2.-Beta.-Pinene
2. Gamma-Terpinene,

Gamma-Terpinene or 1, 4-p-mentadiene or g-terpinene, is an organic compound classified as branched unsaturated hydrocarbons containing one or more unsaturated carbon atoms, and aliphatic branches. Gamma-terpinene as exhibited in Figure 4, is considered to be an isoprenoid lipid molecule. Gamma-Terpinene is liquid and insoluble and relatively neutral. Gamma-terpinene is mainly located on cell membranes and is found in eukaryotes. Gamma-Terpinene is a compound that has a bitter taste and is an herb that can be found in sweet potatoes, green peppers, peppermint, and turmeric. Gamma-terpinene is one of three monoterpene isomers that differ in the position of their two double bonds (alpha- and beta-terpinene being the other). In gamma-terpinene, the double bonds are in position 1- and 4 of the p-mentane skeleton. These are monoterpene and cyclohexadiene. Gamma-terpinene has an important role as an antioxidant, plant metabolites, and a component of essential oils. Gamma-Terpinene was one of the component from nutmeg oil that showed some antimalarial activity against *Plasmodium falciparum* [17,19].

![Figure 4. Chemical structure of Gamma-Terpinene](image)

3. CROWEACIN or Myristicin

Myristicin, also known as asaricin or myristicin (6ci), is a chemical compound that belongs to a group of organic compounds known as benzodioxoles. It is an organic compound containing a benzene ring fused to one of the dioxol isomers. Dioxols are five-membered unsaturated rings of two oxygen atoms and three carbon atoms. Myristicin exists as a solid and is considered to be practically insoluble (in water) and relatively neutral. Myristicin is mainly located in the cytoplasm of cell. Myristicin is a balsamic, spice, and warm flavor compound that can be found in a number of food items and a potential marker for consumption of this food product. Myristicin is an organic molecular entity. It has a role as a metabolite which showed antioxidant, antibacterial and antifungal activities[8,9,20].

![Figure 5. Chemical structure of Myristicin](image)
4. D limonene
Limonene, (+/-) - is a racemic mixture of limonene and naturally occurring cyclic monoterpenes. D-limonene has chemo-preventive and anti-tumor activity through inhibition of p21-dependent signaling. These compounds also induce apoptosis via the induction of the transforming growth factor beta signaling pathway. Thereby inhibiting the modification of post-translational signal transduction proteins. Thus it will result in the termination of the G1 cell cycle and cause the differential expression of genes related to the cell cycle and apoptosis. Limonene is a monoterpane which is a cyclohex-1-ene substituted by a methyl group at position 1 and a prop-1-en-2-y1 group at position 4 respectively. D limonene was one of the important compound in nutmeg oil which showed antioxidant, antimicrobial antiangiogenic activities[9,17,18].

![Figure 6. Chemical structure of D limonene](image6.png)

5. beta.-Myrcene
Myrcene, which has another name beta-myrcene or alpha-myrcene, is an acyclic monoterpenoid. myrcene is an organic compound that is included in the isoprenoid lipid molecule. Myrcene as shown in Figure 7, is a balsamic, must, and peppery tasting compound that can be found in limes and cumin. Myrcene is a yellow oily liquid with a pleasant odor. Flash point is below 200 °F. The compound is insoluble in water. Beta-myrcene is a monoterpane which is an octa-1,6-diene substituent containing methylene and methyl at positions 3 and 7, respectively. Myrcene is a plant metabolite, an agent anti-inflammatory, antimicrobial, antimalarial activity against Plasmodium falciparum D6, anabolic agents, fragrances, flavoring agents and pain relief [17,21]

![Figure 7. Chemical structure of Myrcene](image7.png)

6. Delta.3-Carene
(-) - 3-Carene, has another name car-3-ene or isodiprene. These organic compounds are known as bicyclic monoterpenoids which contain exactly 2 rings attached to each other. Thus (-) - 3-carene is an isoprenoid lipid molecule. The compound (-) - 3-Carene is considered to be a molecule that is insoluble in water) and relatively neutral. (-) - 3-carene in the cell mainly can be found in the membrane and cytoplasm. Carene is a colorless liquid and has a sweet smell like turpentine. (-) - 3-carene will float on the water. (-) - car-3-ene has a chemical structure car-3-ene (3,7,7-trimethylbicyclo [4.1.0] hept-3-ene) with R configuration in position 1 and configuration S at position 6 It is the enantiomer of a (+) -
car-3-ene. The essential oil of nutmeg leaves and fruits showed larvicidal tests against *Aedes aegypti* (L.) (Diptera: Culicidae) [17,22]. The chemical structure of (-) - 3-carene is shown in Figure 8.

![Figure 8. Chemical structure of (-) - 3-Carene](image)

Maluku, Aceh, Sulawesi, Java, Sumatera, and Papua are the main producing areas for nutmeg essential oil in Indonesia. Their consecutive production area were 74,535 Ha; 6,754 Ha; 48,500 Ha; 1,606 Ha; 4157 Ha; and 15,335 Ha [11]. The GC-MS analysis of main component of essential oil of nutmeg from some of these areas showed variety result on their chemical compound in seed with comparison of leaf nutmeg oil as summaries in Table 2 [20,23,24]. The compound that was similar in this comparison among seed and leaf is myristicin with concentration almost 2-7 times smaller than other area. Other research supported this result in showed that concentration of myristicin in seed in the same area was four times bigger than in leaf [15]. Some research showed that the main compounds of nutmeg oil in a variety part of the plant were consist of sabinin (20.2%), terpinen-4-ol (12.1%), safrole (10.3%), α-pinene (9.7%), β-phellandrene (6.6%), and γ-terpinene (5.9%) [8,9]. Comparison of the dominant chemical compound of nutmeg oil from several region in Indonesia region showed that the main components of *M. fragrans* from seed were almost similar which consist of myristicin, terpineol, and terpinolene. Most of chemical compound found from the differences part from the nutmeg plants like root, bark, fruit, mace, leaf and seeds were less and quantitative. The contents of all the nutmeg oil compounds in various parts of the plant are in order of predominance, mace > seed nutmeg > leaf > pericarp[15]. Some compounds in one region, with the same methods of extraction were not detected in different region. As can be seen in Table 2 and from other studies, the greatest diversity of volatile substances about 58 components were found in the leaf and in the mace with 55 components, followed by the nutmeg (53 components) and pericarp (49 components). Comparing with another country, nutmeg oil from Malaysia, India, China showed similar main component but exhibited lower concentration of myristicin than Indonesia [15].

**Table 2.** Comparison of several primary compound of Nutmeg Oil in seed from several region in Indonesia with leaves nutmeg oil from Batang

| West Java | Aceh | Maluku | Batang |
|-----------|------|--------|--------|
| %         | %    | %      | %      | %      |
| Chemical Substances | Chemical Substances | Chemical Substances | Chemical Substances |
| 22,22 | Myristicin | 34,85 | Myristicin | 13,76 | Myristicin | 5.26 | Croweacin/Myristicin |
| 14,15 | 4-Terpinol | 33,00 | Alpha-Terpinol | 1,54 | terpinolene | 2.64 | .Alpha.-Terpinolene |
| 6,98 | Sabinena | 14,56 | Terpinol-4, | 15,30 | pinena | 30.28 | .gamma.-Terpinene |
| 5,45 | α-Pinena | 2,38 | safrole, | 10,22 | osime | 34.46 | 2.-Beta.-Pinene |
| 3,88 | Δ Limonena | 1,86 | Terpinol 1-ol | 1,76 | eukaliptol | 3.20 | .beta.-Myrcene |
| 7,1 | 2-b-Pinena | 2,44 | safrole | 3.15 | .Delta.3-Carene |
|       |       | 3,42 | mirse | 2.73 | .beta.-Phellandrene |
|       |       |       |   | 5.19 | dl-Limonene |
The comparison of the content of myristicin compounds as shown in Table 4 show that nutmeg from Aceh has the highest myristicin content. Furthermore, the other major compounds are Terpineol, terpinolene, pinena, and limonena. Apart from these chemical compounds, each region has different chemical compounds between regions. Several compounds were identified in nutmeg and mace of the nutmeg plant with terpinen-4-ol, β-pinene, and limonene being the dominant constituents common to volatile oil in all species [25]. The terpinen-4-ol, γ-terpinene and α-terpinene, were also the three main compounds of tea tree oil (Melaleuca alternifolia) [26].

The difference of chemical compound between each region is generally determined by species (genetic factors), source, plant age, growth condition, ecological conditions (climate and soil fertility), the extraction method used, the cultivation practices, harvesting process, geographic environment and the other external factors [2,8,9]. The other process affecting the chemical compound was distillation time and boiling method[2]. Considering the variation and concentration of the myristicin and other chemical compound in nutmeg oil, the result show that the quality of nutmeg oil from Batang region is showed the characteristic component and variation of chemical compound which is suitable for application in industrial purposes.

4. Conclusion
The characterization of chemical composition nutmeg oil from Batang Region showed gamma.-Terpinene, 2-beta.-pinene, linalool L, Myristicin, dl-Limonene, .beta.-Myrcene, .delta.-3-Carene, .beta.-Phellandrene and .alpha.-terpinolene as major substances. Analyzing essential oil from nutmeg containing desired characteristics will offer best suites the needed industrial applications.

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