Characterization of citronella grass essential oil of *Cymbopogon winterianus* from Batang region, Indonesia

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Abstract. The needs of herbal medicine tend to increase because of their little side effect and complications. Citronella oil has been under an attention due to progressive need for new biochemical effects to establish a novel production from this essential oil. The chemical properties of essential oil of Citronella grass from Batang Indonesia, distilled from leaves and shoots of *Cymbopogon winterianus* were found to be within the specifications set by Indonesia National Standard for Essential Oil. The result of GC-MS analysis showed 30 component with the major components of essential oil: Isopulegol, Tricyclo[5.2.1.0(1,5)]decan, LINALOOL L, Butanoic acid, Citronellyl acetate, Geranyl acetate, and Elemol. Comparison with commercial citronella oil showed different concentrations in the essential oil of Citronella grass from Batang Indonesia. Essential oil of Citronella grass from Batang Indonesia was also showed antimicrobial and dermatophic activity according to PASS analysis.

1. Introduction

Citronella grass plant is one of the producers of essential oils where Indonesia is one of the largest producers of essential oils in the world. At present there are around 40 types of essential oils, of which 20 have high economic value markets[1]. Essential oils from plants are produced through the process of distillation of roots, bark, leaves, flowers and seeds[2]. One of the plants that produce essential oils is Citronella grass (*Cymbopogon winterianus* or *A. nardus* Java de Yong), Maha Pengiri type [3]. Citronella Oil from Central Java was known as Citronella Oil of Java in the trading world. The role of fragrant Citronella grass is very large as a commodity of foreign exchange and regional income as well as employment. The production of fragrant lemongrass oil in Indonesia is produced one of them from the Batang area of Central Java. Citronella grass had a high prospective as essential oil commodity. Demand for citronella oil is quite high and the price is stable and tends to increase. Cultivating Citronella grass is quite easy and these plants can live on marginal lands. Demand for citronella oil is quite large where the market needs always increase 3 - 5% per year. The purpose of this study was to determine the quality of citronella oil from the Batang area by looking at the characterization of
chemical composition contained therein using the GCMS method and its potential use for medicinal purposes.

2. Material and methods

2.1. Study area
Citronella grass was collected from the Blado area. The area for sample collection is illustrated in Figure 1. Lemongrass oil is produced through the distillation process of Citronella grass leaves. Previously the leaves were dried for 24 hours to accelerate and ease oil expenditure. The dried leaves will be directly refined without going through the storage process to get a good quality of Citronella oil.

2.2. Citronella oil extraction
The extraction method was carried out using C. winterianus dried leaves. The distillation of Citronella grass oil is done by steaming Citronella grass leaves. Kettle made of stainless steel. Steam will come out of the kettle, then flow through a pipe connected to the condenser as a cooler. Steam containing a mixture of water and oil will drip at the end of the pipe and collected in a container. Then the separation process is carried out to obtain pure Citronella oil.

2.3. GC-MS analysis
Gas chromatography-mass spectroscopy (GC-MS) technique was used to analyze the chemical composition of Citronella oil. The lemongrass oil was separated using a capillary column measuring 30 m x 0.25 mm (I.D. i.d.) coated with a 0.25 mm phenylmethyl siloxane 5% film at a column temperature of 80 ° C for injection and fitted to flame ionization detector (FID). The programming temperature is set at 10 ° C min-1 to 150 ° C and then continued from 5 ° C min-1 to 250 ° C and ends from 10 ° C min-1 to 280 ° C and lasts for 5 minutes. Helium is used as a carrier gas with a flow rate of 1 mL / min using splitless injection, (2μL). The spectrometer is operated using the electron-impact mode (EI), with an electron energy of 70 eV and a scanning range of 50-550 amu. The temperatures for inlet and ionization sources are 240 ° C and 280 ° C, respectively. Identification of each component of lemongrass oil is done through comparison of retention times with standard substances.

Figure 1. Location of Blado in Batang regency
and also by matching mass spectral data with MS and reference libraries (NIST and Wiley 275.l). the percentage of area obtained by FID is used as a basis for quantitative analysis purposes [4].

2.4. Determination of physico-chemical substances in Citronella oil

Determination of physicochemical substances in lemongrass oil is done by using chemical compound analysis using Pubchem and Pubchem Bioassay facilities in NCBI [www.ncbi.nlm.nih.gov] to search for Canonical SMILES (molecular-input pathway entry system simple) and pharmacy expert program [5].

2.5. Prediction of activity spectra for citronella oil substances using PASS approach

PASS (Activity Spectrum Prediction for Substances) is used to evaluate the general biological potential of molecules such as organic drugs in citronella oil. This software is used to predict various types of biological activity based on the structure of organic compounds in citronella oil, so that it can estimate the biological activity profile for virtual molecules, before chemical synthesis and biological testing. The Biological Activity Spectrum of chemical compounds produced by the lemongrass oil is a collection of various types of biological activity that reflects the results of the interaction of compounds with various biological entities. Pa (probability "to become active") is used to estimate the possibility that the compound under study is included in the sub-class of active compounds (resembling molecular structures, which are most typical in the "active" sub-set in the PASS set)[6][7][8].

3. Result and discussion

The results of GC-MS analysis of the essential oils are presented in Table 1. The chemical composition of citronella oil was consist of 30 compounds. The main component of citronella oil are (-)-Isopulegol (47.43% and 14.28%), as shown on Table 1. The chromatogram of citronella oil was illustrated in Figure 2. The result was in contrast with previous research which found essential oil of C. winterianus from Belgrade contained mainly citronellal (27.00%), trans-geraniol (22.78%), and citronellol (10.09%) [9], whereas essential oil of C. winterianus from India contains geraniol, citronellal, and citronellol [10].

| No | RTime | Area % | Chemical Substances                  |
|----|-------|--------|-------------------------------------|
| 1  | 7.465 | 0.10   | .beta.-Myrcene                      |
| 2  | 9.145 | 0.10   | 1-Phellandrene                      |
| 3  | 9.282 | 6.52   | Tricyclo[5.2.1(1,5)]decane          |
| 4  | 9.734 | 0.15   | cis-Ocimene                         |
| 5  | 12.056| 0.11   | .ALPHA.-TERPINOLENE                |
| 6  | 12.450| 1.53   | LINALOOL L                          |
| 7  | 14.309| 1.20   | ISOPULEGOL 2                        |
| 8  | 14.860| 47.43  | (-)-Isopulegol                      |
| 9  | 14.955| 0.43   | ISOPULEGOL 1                        |
| 10 | 15.140| 0.12   | Isogeraniol (CAS)                   |
| 11 | 18.073| 8.45   | Butanoic acid, 3,7-dimethyl-6-octenyl ester (CAS) |
| 12 | 19.144| 14.28  | (-)-Isopulegol                      |
| 13 | 22.172| 0.82   | Phenol, 2-methoxy-3-(2-propenyl)- (CAS) |
| 14 | 22.607| 2.31   | Citronellyl acetate                 |
| 15 | 23.543| 3.46   | Geranyl acetate                     |
| 16 | 23.860| 0.14   | Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- |
| 17 | 24.120| 0.77   | .BETA. ELEMENE                      |
| 18 | 25.985| 0.09   | .alpha.-Humulene (CAS)              |
| 19 | 26.844| 2.36   | GERMACRENE-D                        |
| 20 | 27.210| 0.23   | Torreyl                             |
Identification of major chemical compound from essential oil of *C. winterianus* by GCMS analysis:

1. Isopulegol

Isopulegol as illustrated in Fig. 3 showed retention time 14,860, Mass Peak 335. Raw Mode : Averaged 14,855-14,865(2352-2354), base Peak 121,9. Molecular formula C₁₀H₁₈O. Chemical names, Isolpulegol; (-)-Isopulegol; l-Isopulegol. Molecular Weight 152.25 g/mol. Isopulegol is a p-menthane monoterpenoid. It has a role as a metabolite. Commonly (-)-Isopulegol is isolated from *Mentha pulegium* and other essential oil.

**Figure 2.** Chromatogram of citronella oil

**Figure 3.** Chemical Structure depiction of Isopulegol (pubchem.ncbi.nlm.nih.gov)(left) and peak (right)
2. Citronelil acetate. Citronelil acetate in Fig 4. showed retention time 22,605, Raw Mode: Averaged 22,600-22,610 (3901-3903). Molecular formula C_{12}H_{22}O_{2}. Chemical names: Citronellyl acetate; Citronellol acetate; 3,7-Dimethyloct-6-en-1-yl acetate; 3,7-Dimethyl-6-octen-1-yl acetate. Molecular Weight 198.3 g/mol. Isopulegol is a p-menthane monoterpene. It has a role as a metabolite. (+)-Isopulegol is isolated from *Mentha pulegium* and other essential oil. Citronellol acetate is a monoterpene that is the acetate ester of citronellol. It has been isolated from *Citrus hystrix*. It has a role as a plant metabolite. It is an acetate ester and a monoterpene. It derives from a citronelol. (+)-Citronellyl acetate is a constituent of citrus oil, orange juice, lemon juice and peel, grapefruit peel, swangi (*C. hystrix*), ginger, tarragon, myrtle leaf, West Indian lemongrass oil and beer.

![Figure 4. Chemical structure depiction of citronelil acetate (pubchem.ncbi.nlm.nih.gov)(top) and peak (right)](image)

3. Citronellyl
Citronellyl that exhibited in Fig 5. showed retention time 18,075. Molecular formula C_{14}H_{26}O_{2}. Averaged 18,070-18,080 (2995-2997). Chemical names: Butanoic Acid 3,7-dimethyl-6-octenyl ester (CAS), Citronellyl butyrate, Citronellyl, Citronellyl n-butyrate, natural rhodinol, butylated. Molecular Weight 226.35 g/mol. Citronellyl butyrate is found in citrus. Citronellyl butyrate is a constituent of Ceylon citrus oil, tomato, orange juice and passion fruit juice. Citronellyl butyrate is a flavouring ingredient.

![Figure 5. Chemical Structure depiction of Citronellyl (pubchem.ncbi.nlm.nih.gov) (top) and peak (right)](image)

4. Tricyclo[5.2.1.0(1,5)]decane 6,52
Figure 6. Chemical Structure depiction of Tricyclo[5.2.1.0(1,5)]decane 6,52 (pubchem.ncbi.nlm.nih.gov) (top) and peak (right)

Tricyclo [5.2.1.0(1,5)] decane 6,52 having retention time 9.280 Raw Mode: Averaged 9.275-9.285(1236-1238). MassPeak 278. Molecular formula C_{10}H_{16}. Chemical names Tricyclo [5.2.1.0(1,5)] decane TRICYCLO[5.2.1.0 1,5] DECANE. Molecular Weight 136.23 g/mol (Figure 6.)

5. Geranyl acetate 3,46

Figure 7. Chemical Structure depiction of Geranyl acetate 3,46 (pubchem.ncbi.nlm.nih.gov) (top) and peak (right)

Geranyl acetate is a clear colorless liquid with an odor of lavender. As illustrated in Figure 7. It has retention time 23.545, Raw Mode: Averaged 23.540-23.550(4089-4091). MassPeak 248. Molecular formula C_{12}H_{20}O_{2}. Chemical names Geranyl acetate 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-(CAS), Geraniol acetate (CAS), Bay pine (oyster) oil, Acetic acid, geraniol ester. Molecular Weight 196.29 g/mol. Geranyl acetate is a monoterpenoid that is the acetate ester derivative of geraniol. It has a role as a plant metabolite. It is an acetate ester and a monoterpenoid. It derives from a geraniol.

6. Germacrene-D 2,36

Figure 8. Chemical Structure depiction of GERMACRENE-D 1,6 (pubchem.ncbi.nlm.nih.gov) (top) and peak (right)
Grmacrene-D 2,36 in Figure 8 showed retention time 26,845, Raw Mode : Averaged 26,840-26,850(4749-4751). MassPeak 299. Molecular formula C_{15}H_{24}. Chemical names GERMACRENE-D 1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-8-(1-METHYLETHYL)-, [S-(E,E)]-GERMACRA-1(10),4(15). Molecular Weight 204,35 g/mol

7. Elemol 2,57

![Chemical Structure depiction of Elemol 257](top) and peak (right)

Elemol has retention time 28,730, Raw Mode : Averaged 28,725-26,735(5126-5128). MassPeak 284 (Figure 9). Molecular formula C_{15}H_{26}O. Chemical names Elemol, Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]- (CAS), o-Menth. Molecular Weight 222,37 g/mol.Elemol is a sesquiterpenoid that is isopropanol which is substituted at position 2 by a (3S,4S)-3-isopropenyl-4-methyl-4-vinylcyclohexyl group. It has a role as a fragrance and a plant metabolite. It is a sesquiterpenoid, a tertiary alcohol and an olefinic compound.

3.1. Dominant chemical compound of C. winterianus from Batang region

One of the most important components in lemongrass oil is citronellal. These components have high economic value as ingredients in the fragrance, green medicine and pharmaceutical industries. The chemical compound obtained in this study turned out to be very different from the results obtained by other researchers [9][11], but supported by Andila et al. [12], in finding isopulegol as dominant chemical compound in C. winterianus. Lots of research in Indonesia have made various efforts to convert citronellal into isopulegol due to its high economic value [13][14][15].Isopulegol as dominant chemical compound is a monoterpen alcohol intermediate in the preparation of (-)-menthol(C_{10}H_{20}O). Isopulegol is an intermediate compound as basic material made from citronellal. Isopulegol conversion from citronellal are carried out through cyclization reactions [16]. Furthermore, isopulegol will be converted into menthol through a hydrogenation reaction (Figure 10). This reason was made Isopulegol became a material with high demand in the industry.Isopulegol is used in the manufacture of menthols with the character of the smell and taste of peppermint which is widely used in the pharmaceutical industry, the soap industry and toothpaste. The (-)-isopulegol can be physically separated and directly hydrogenated to (-)-menthol, while the remaining isopulegol isomers are recycled to an equilibrium mixture. Alternatively, the total isomeric mixture of isopulegols can be hydrogenated to an isomeric menthol mixture. (-)-Menthol is separated and purified through high efficiency distillation columns and recrystallization (of derivatives). The remaining isomeric menthols can be isomerized catalytically to an equilibrium mixture favoring the desired(-)-menthol isomer.
Compared to the previous work [17], the oil content of the extracted citronella oil from *C. nardus* is the same as from *C. winterianus* extracted in Batang Indonesia, but different with in Citronella grass from other regions [4][11][18]. Castro *et al.* [17] found that Citronella grass leaves have 3 main compounds which are citronellol, geraniol and elemol. Citronellol and Elemol, which is the main compound found in the citronella oil extracted from *C. nardus* leaves in Tocantins State and *C. winterianus* Lombok was also found in the extracted citronella oil from *C. winterianus* leaves grown in Indonesia, but was not found in Malaysia. This might be affected by several factors such as climate, age of plantation, harvesting time and efficiency of distillation method [12][18].

### 3.2. Medicinal activities and therapeutic properties of essential oils from *C. winterianus* from Batang region

The medicinal activities and therapeutic properties of essential oils of *C. winterianus* from Batang region as exhibited in Table 2, are correlated with their chemical composition. Plants contain various substances naturally that can cure various diseases suffered by humans.

In Indonesia, the plant has been used by the community for traditional medicines. There are several substances in the medicinal plant with different activities that can work individually or together in treating a disease. Citronella oil is traditionally known for its natural insect repellent activities and also had many uses as aromatherapy [4]. The citronella oil from Batang region, based in PASS test prediction, showed some medicinal activities and therapeutic function. Surprisingly, almost all of the dominant chemical compound showed dermatological activities, antimicrobial, antiinflammatory, antieczematic, antipsoriatic and antiviral, beside other pharmacological activities. This result was in accordance with [9][12][19][20][21][22].

**Table 2.** The medicinal activities and therapeutic properties of essential oils of *C. winterianus* from Batang region

| Activity            | Isopulegol | Citronellol acetate | Tricyclo[5.2.1.0(1,5)]decane | Cytronellyl acetate | Geranyl acetate | Germacrene-D | Elemol |
|---------------------|------------|---------------------|------------------------------|--------------------|-----------------|--------------|--------|
| Dermatologic        | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antiinflammatory    | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antieczematic       | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antipsoriatic       | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antiviral           | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antifungal          | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antiprotozoal       | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antipruritic        | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
| Antimycobacterial   | ✓          | ✓                   | ✓                            | ✓                  | ✓               | ✓            | ✓      |
The majority of biologically active compounds from Citronella oil potencies from Batang region exhibited a wide spectrum of different effects. Some of them are useful in treatment of human diseases. The combination of GC-MS methods and in silico methods using PASS program revealed more comprehensive result for the improvement of Citronella oil potency from Batang region for further improvement to achieve the industrial needs.

4. Conclusion
The characterization of chemical composition Citronella oil showed Isopulegol, Tricyclo [5.2.1.0(1,5)] decane, LINALOOL L, Butanoic acid, Citronellyl acetate, Geranyl acetate, and Elemol as major substanties. The potency of Citronella oil from Batang region was potentially use for medicinal and therapeutic activities. This result had showed its potency to improved further for industries.
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