GC-MS analysis and antimicrobial activity determination of *Citrus medica* L. var proper leaf essential oil from South Sulawesi against skin pathogen microorganism

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**Abstract.** A research about *Citrus medica* L. var sarcodactylis had been conducted and it showed a significant antimicrobial activity, thus drive our curiosity to investigate the other variety from the same species, *Citrus medica* L. var proper. This research focuses in chemical compound study and antimicrobial activity screening against *Staphylococcus aureus*, *Propionibacterium acne*, and *Candida albicans* of *Citrus medica* L. var Proper leaves’ essential oil. The essential oil is distillated from fresh leaves by hydrodestillation. The chemical compound was analysed using GC-MS instrument while the antimicrobial activity was tested using disk diffusion method. The results showed that the major component of the essential oil was Z-citral, citral and limonene compounds. The antimicrobial activity test results against the test microorganism are 9.15±0.15 mm, 11.15±1.3 mm and 8.02±0.48 mm, consecutively, for *Staphylococcus aureus*, *Propionibacterium acne* and *Candida albicans*.

**Keywords:** *Citrus medica* L. var Proper, antimicrobial, extract, essential oil

1. **Introduction**

Fruits from Rutaceae family (Citrus) are one of the word most produced fruits. Citrons are among the less known member of the family, which include oranges, mandarins, lemons and grapefruit, while they were the first fruits of the genus that reach Mediterranean region [1]. *Citrus medica* was a named given to *Citron* by Linnaeus possibly because its importance in pharmaceutical usage of the genus that time [2]. In general, *Citrus medica* L. had been used widely as traditional medicine and can be found in various traditional medicine literatures such as Ayurveda [3]. This plant belong to several variety like ‘diamante’, ‘corsican’, ‘poncire commun’, ‘sarcodactylis’ and ‘rhobs-arsa’ [1]. *Citrus media* L. var Proper is one of the variety that can be found in Indonesia and known for its empirical application in traditional medicine.

Investigation about antimicrobial activity of various Citrus plants had been conducted. A previous study shows that ethyl acetate extracts from various fresh and dried citrus peels were active against *Staphylococcus aureus* and *Escherichia coli* [4]. Another investigation focusing in antimicrobial activity of *Citrus medica* L. var sarcodactylis leaves’ essential oil confirm its activity against *S. aureus*, *B subtilis*, *M. luteus* and *E. coli* with inhibition zone diameter 11±0.3 mm, 15±0.8 mm, 11±0.3 mm and 8±0.7 mm, consecutively [5]. Based on these findings, this research aimed to
investigate further about antimicrobial activity form another variety of *Citrus medica* L., which is ‘proper’ variant as well as its physicochemical and phytochemical properties.

2. Materials and Methods

2.1. Plant material and authentication

*Citrus medica* L. var Proper leaves were collected from Makassar, South Sulawesi, Indonesia. The plant specimen was authenticated by Biology Department, Hasanuddin University.

2.2. Instrumentation

GC-MS-QP2010 Ultra Shimadzu is used in this study and the mass spectra database is used as comparison to identify the constituents.

2.3. Distillation

The fresh sample was subjected to modified hydrodistillation method with minimum water usage until the essential oil no longer appears in the condensate. The oil then dried with anhydrous sodium sulphate and stored in airtight container at 4°C [5].

2.4. GC-MS Analysis

The acquired essential oil was analyzed using GC-MS instrument with the standard protocol. Column condition: Rxi®-1 ms restech, 30x0.25 mm ID, 0.25 µm, column temperature 60°C, injection temperature 250°C, interface temperature 300°C. Helium was used as the carrier gas and dimethyl polysioxane is used as the stationary phase. One microliter of essential oil was diluted up to 2 mL with dichloromethane and the total runtime was 50 minutes. All the mass spectrum acquired was compared with the data base.

2.5. Antimicrobial activity determination

Essential oil was tested against *Staphylococcus aureus*, *Propionibacterium acne* and *Candida albicans* using disk-diffusion method. The bacteria were cultivated on Nutrient Agar (Merck) while the yeast was grown in Potato Dextrose Agar (Merck) plate. Into a standard 6 mm blank disc (Oxoid), 20 µL samples were introduced with a micropipette and directly put onto the agar surface after sample introduction. All the plate then incubated in their respective temperature for 24 hours before inhibition zone diameter measurement. Standard antibiotic disks (Ampicillin and Nystatin) are used as positive control [6].

3. Results and Discussion

3.1. Chemical constituent of the essential oil

The GC-MS results showed that there are 17 peaks (Figure 1) that indicates that amount of compound presence in the essential oil with the major component of the essential oil was Z-citral and citral compound followed by dL-limonene and cyclohexane compounds. The detail was shown in the Table 1.
Table 1. Chemical components of the essential oil

| No. | Name                                      | RT  | Area % |
|-----|-------------------------------------------|-----|--------|
| 1   | 6-Methyl-5-hepten-2-one-β -myrcene         | 6.962 | 1.74   |
| 2   | β-Myrcene                                 | 7.047 | 1.28   |
| 3   | dL-Limonene                               | 8.135 | 11.54  |
| 4   | Cyclohexene, 1-methyl-4-(1-methylethenyl)  | 8.324 | 11.47  |
| 5   | dL-Limonene                               | 8.504 | 8.41   |
| 6   | 1,3,6-Octatriene,3,7 dimethyl-, (E)- (cas) | 8.720 | 0.96   |
| 7   | Nonanal (cas) n-nonanal                   | 10.254 | 0.63  |
| 8   | Linalool                                  | 10.441 | 0.84  |
| 9   | Citronella                                | 11.850 | 1.40  |
| 10  | Phellandral                               | 12.158 | 0.59  |
| 11  | trans-Caran, 4,5 epoxy                     | 12.752 | 0.87  |
| 12  | Z-Citral                                  | 15.170 | 23.44 |
| 13  | Citral                                    | 16.129 | 28.18 |
| 14  | Undecanal (cas) hendecanal                | 16.991 | 0.45  |
| 15  | Neryl acetate                             | 18.934 | 1.47  |
| 16  | Geranyl acetate                           | 19.582 | 6.00  |
| 17  | 2-hexadecen-1-ol,3,7,11,15-tetramethyl-  | 39.015 | 0.73  |

Table 2. Antimicrobial activity test results

| Test microorganism   | Inhibition zone (mm) |
|----------------------|-----------------------|
|                      | Citrus medica L. essential oil | Positive control |
| *Staphylococcus aureus* | 9.15±0.15              | 15.27±2.43       |
| *Propionibacterium acne* | 11.15±1.33             | 13.29±3.21       |
| *Candida albicans*    | 8.02±0.48              | 7.57±0.11        |

3.2. Discussion

Various parts of Citrus medica L. plants has been used traditionally to treat illness [7–9]. Citrus medica L. belong to Rutaceae family which is known, used and exploited for its essential oil content [10]. Essential oil (also known as volatile oil) from plants are known to possess antimicrobial activities [11].

The GC-MS analysis of the essential oil showed that the major components are citral, Z-citral and limonene compounds. A study reports a similar results about chemical components of Citrus medica L. leaves essential oil with limonene and citral as the major constituents [12]. The amount of limonene is somewhat low compared to the amount recovered from Citrus medica L. var Diamante with the same method [13]. The variation of volatile compound production of a plant can be affected by the geographical, environmental and physiological factors [14].

The antimicrobial activity maybe contributed by to the presence of limonene [5] and citral [15] in the essential oil. In the other hand, a study also reported a contradictory result about antifungal effect of citral containing essential oil [16]. There is a study which indicates that limonene and its enantiomers have antimicrobial properties against eight different test pathogens. The study reveals that stereochemistry play an important role in the effect of biological active essential oil which sometimes short-sighted. The same study also shows the possible antagonistic activity of chemical components of essential oil [17]. As a comparison, a research also reported that essential oil form Citrus medica L. var sarcodactylis possess antibacterial activity against S. aureus and B. subtilis with inhibition zone.
diameter 11±0.3 mm and 15±0.8 mm, respectively. The major components of the essential oil are limonene and citral (in the form of neral and geranial) [5].

A study that involve various citrus plants (orange, lemon and mandarin) shows that the antimicrobial activity of the essential oil may also be affected by the interaction of various chemical constituents of the oils (synergistic, additive or antagonistic effects) [18]. Another research indicate that plant extract from various parts of the Citrus medica L. plant also have antimicrobial activity [19].

4. Conclusions
Essential oil from Citrus medica L. var proper fresh leaves has antimicrobial activity against S. aureus, P. acne and C. albicans. The major components of the essential oil are limonen and citral compounds.

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