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

Aliyah, A Himawan, H Rante, Mufidah, D R Ningsih

Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia

E-mail: himawan@unhas.ac.id

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 C*itron* 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-el-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 subtillis, 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

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

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.

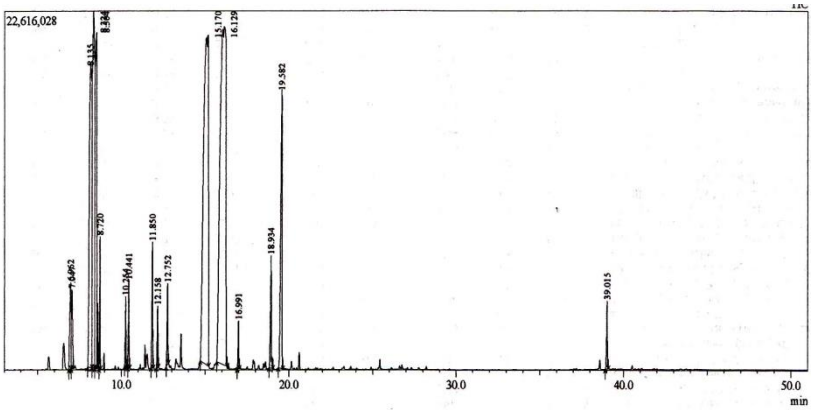

Figure 1. Chromatogram profile of the essential oil.

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-[R[R*R*-(E]]](cas) phytol	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, addictive 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.

Acknowledgement

The author would like to thank to Ministry of Research, Technology and Higher Education as the institution who grant this research through PUPT 2017 scheme.

References

[1] Tomi Â, Bessiere J, Casanova J, Lota M and Serra DDR 1999 Chemical composition of peel and leaf essential oils of *Citrus medica* L. and *C. limonimedica* Lush. *Flavour Fragr. J.* **166**(14) 161–6.

[2] Mabberley D J 2004 Citrus (Rutaceae): A review of recent advances in etymology, systematic and medical application. *BLUMEA* **49** 481–98.

[3] Panara K, Nishteswar K and Joshi K. A 2012 Review on phytochemical and pharmacological properties of citrus medica. *Int. J. Pharm. Biol. Arch.* **3**(6) 1292-97.

[4] Chanthaphon S, Chanthachum S and Hongpattarakere T 2008 Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. Songklanakairin *J. Sci. Technol.* **30** 125–31.

[5] Theanphong O, Songsak T and Mingvanish W 2008 Chemical composition and antimicrobial activity of the essestial oil from *Citrus medica* L . var . sarcodactylis (Sieber) Swingle Leaf. *Mahidol Univ. J. Pharm. Sci*. **35**(1–4) 57–61.

[6] Tavman A 2009 Antimicrobial activity of turkish citrus peel oils. *Pak. J. Bot.* **41**(6) 3207–12.

[7] Ahmed F 2017 Antiulcer activity of aqueous extract of *Citrus medica* Linn. fruit against ethanol-induced ulcer in rats *Advan. Biol. Res.* **6**(1) 24-29.

[8] Zhao P, Duan L, Guo L, Dou L L, Dong X, Zhou P, Li P and Liu E H 2015 Chemical and biological comparison of the fruit extracts of *Citrus wilsonii* Tanaka and *Citrus medica* L Article in Food Chemistry *Food Chem.* **173** 54-60.

[9] Sah AN, Joshi A, Juyal V and Kumar T 2011 Antidiabetic and hypolipidemic activity of *Citrus medica* Linn. seed extract in streptozotocin induced diabetic rats. *Pharmacogn J.* **3**(23) 80-84.

[10] Chutia M, Bhuyan PD, Pathak MG, Sarma T C and Boruah P 2009 Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. *LWT-Food Sci. Technology* **42** 777–80.

[11] Solórzano-santos F and Miranda-novales MG 2015 Essential oils from aromatic herbs as antimicrobial agents. *Curr. Opin. Biotechnol.* **23**(2) 136–41.

[12] Bhuiyan M N I, Begum J, Sardar P K and Rahman M S, Cantonment POC 2009 Constituents of peel and leaf essential oils of *Citrus medica* L. *J. Sci. Res.* **1**(2) 387–92.

[13] Tundis R, Calabria U, Bonesi M, Calabria U and Calabria U 2011 Chemical composition and bioactivity of *Citrus medica* L. cv . Diamante essential oil obtained by hydrodistillation. *Nat. Prod. Res.* **25**(8) 789-99.

[14] Figueiredo A C, Barroso J G, Pedro L G and Scheffer J J C 2008 Factors affecting secondary metabolite production in plants: Volatile components and essential oils factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Fragr. J.* **23** 213–2261.

[15] Rojas-graü M A, Martin-belloso O, Mchugh T and Rojas-grau M A 2017 Effect of plant essential oil compounds on mechanical barrier and antimicrobial properties of alginate-apple puree edible films barrier and antimicrobial properties of alginate – apple puree edible films. *J. Food Eng.* **81**(3) 634-641.

[16] Nakahara K, Alzoreky N S and Yoshihashi T 2003 Chemical Composition and Antifungal Activity of Essential Oil from *Cymbopogon nardus* (Citronella Grass). *JARQ* **37** 249–52.

[17] Vuuren S F van and Viljoen A M 2007 Antimicrobial activity of limonene enantiomers and 1 , 8-cineole alone and in combination Antimicrobial activity of limonene enantiomers and 1 , 8-cineole alone and in combination *Flavour Fragr. J.* **22** 540–4.

[18] Espina L, Somolinos M, Lorán S, Conchello P, García D and Pagán R 2011 Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes *Food Control.* **22**(6) 896–902.

[19] Sah A N, Juyal V and Melkani A B 2011 Antimicrobial activity of six different parts of the plant. *Pharmacogn. J.* **3**(21) 80–3.