



## Research Article

## Antimicrobial and Volatile Compounds Study of Four Spices Commonly Used in Indonesian Cullinary

Alwani Hamad<sup>1</sup>, M. Gigih Panji Mahardika<sup>1</sup>, Istifah<sup>1</sup>, Dwi Hartanti<sup>2,\*</sup>

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, University of Muhammadiyah Purwokerto, Jl. Raya Dukuwaluh PO Box 202, Banyumas, Central Java, Indonesia 53182

<sup>2</sup>Faculty of Pharmacy, University of Muhammadiyah Purwokerto, Jl. Raya Dukuwaluh PO Box 202, Banyumas, Central Java, Indonesia 53182

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## E-mail address:

dwhartantihamad@gmail.com

## ABSTRACT

The n-hexane extracts of the aerial parts of *Ocimum x citriodorum* and the leaves of *Cymbopogon citratus*, *Syzygium aromaticum* and *Syzygium polyanthum* were evaluated for their antimicrobial activity against some food borne microorganisms. Their volatile compounds were analyzed by gas chromatography/mass spectrometry (GC/MS). All extracts inhibited the growth of *Bacillus subtilis*. The extract of *S. polyanthum* showed the strongest inhibitory activity against *Salmonella typhimurium*. The growth of *Staphylococcus aureus*, *Escherichia coli* and *Vibrio cholera* were not inhibited by all extracts. The major volatile constituent of *O. x citriodorum*, *C. citratus*, *S. aromaticum* and *S. polyanthum* crude extracts were citral, germacrene D-14-ol, p-eugenol and squalene, respectively.

Keywords: antimicrobial, *Cymbopogon citratus*, n-hexane extracts, *Ocimum x citriodorum*, *Syzygium aromaticum*, *Syzygium polyanthum*, volatile chemical constituents

### 1. Introduction

Foodborne illness is a growing public health problem in developing as well as developed countries (Jahan, 2012). In Indonesia, paratyphoid is one of common foodborne illness along with food poisoning caused by *Escherichia coli*, *Vibrio cholera*, *Bacillus subtilis*, and *Staphylococcus aureus* (Vollaard et al., 2004). Plant derived products are often considered to be more natural and safer alternatives to chemicals and thus have become popular in the scope of searching novel antimicrobial agents for food and pharmaceutical applications (Han and Bhat, 2014).

Spices have been reported possessing antimicrobial activity (Babu et al., 2011; Joe et al., 2009; Panpatil et al., 2013; Rahman et al., 2010; Sethi et al., 2013; Shan et al., 2007; Vaishnari et al., 2007). *Ocimum x citriodorum* (lemon basil, Indonesian name: kemangi), *Cymbopogon citratus* (lemon grass, Indonesian name: sereh) *Syzygium aromaticum* (clove, Indonesian name: cengkih) and *Syzygium polyanthum* (bay leaf, Indonesian names: salam) are four spices commonly used in Indonesian cullinary. Previously, *O. x citriodorum* was reported active as antimicrobial against *Staphylococcus aureus*, *S. epidermis*, *Streptococcus mutans*, *Lactobacillus*

*casei*, *Listeria ivanovii*, *L. monocytogenes*, *Enterococcus faecalis*, *E. faecium*, *Escherichia coli*, *Proteus mirabilis*, and *Candida albicans* (Carovic-Stanko et al., 2010; Maryati et al., 2007; Thaweboon and Thaweboon, 2009). *C. citratus* had antimicrobial activity against *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Desulfovibrio alaskensis*, *Penicillium expansum*, *P. verrucosum*, *Aspergillus flavus*, *A. ochraceus*, and *C. albicans* (Balakrishnan et al., 2014; Bassolé et al., 2011; Khan and Ahmad, 2012; Korenblum et al., 2013; Nguefack et al., 2009; Paranagama et al., 2003). *S. aromaticum* essential oil had been known widely as a potent antimicrobial against various bacteria and fungi (Hossain et al., 2014; Joshi et al., 2010; Naveed et al., 2013; Oliveira et al., 2013; Pandey and Singh, 2011; Park et al., 2007; Pinto et al., 2009). *S. polyanthum* possessed antimicrobial activity against *B. subtilis*, *B. cereus*, *P. aeruginosa*, *Salmonella thypi*, *E. coli*, *Tricophyton mentagrophytes* and *C. albicans* (Cornelia et al., 2005; Dewanti and Wahyudi, 2011; Kusuma et al., 2011; Murhadi et al., 2007).

In this study, we evaluated the minimal inhibitory concentration (MIC) and volatile chemical constituents of crude extracts of *O. x citriodorum*, *C. citratus*, *S. aromaticum* and *S. polyanthum* against common foodborne bacteria in Indonesia.

**Table 1.** MIC of crude extracts of spices against tested microorganisms

Microorganisms	MIC ( $\mu\text{g/mL}$ )			
	<i>O. x citriodorum</i>	<i>C. citratus</i>	<i>S. aromaticum</i>	<i>S. polyanthum</i>
<i>B. subtilis</i>	31.25	31.25	125	62.5
<i>E. coli</i>	>1000	>1000	>1000	>1000
<i>S. aureus</i>	>1000	>1000	>1000	>1000
<i>S. typhimurium</i>	1000	1000	>1000	31.25
<i>V. cholera</i>	>1000	>1000	>1000	>1000

## 2. Materials and Methods

### 2.1. Plant materials and chemicals

Fresh aerial parts of *O. x citriodorum* and the leaves of *C. citratus*, *S. aromaticum* and *S. polyanthum* were purchased from the local market at Purwokerto, Indonesia. The plant materials were air dried and pulverized to a fine powder. N-hexane (Sigma-Aldrich) was used as solvent. Nutrient broth (Oxoid) was used as medium for the microorganisms growth.

### 2.2. Extraction

The dried powder of plant materials were extracted by maceration with n-hexane as previously described (Har and Ismail, 2012).

### 2.3. Microbial strains

Five bacterial strains were obtained from the American type culture collection (ATCC; Rockville, MD, USA) as well as the culture collection of the Assessment Service Unit, Airlangga University, Surabaya, Indonesia. They were *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Salmonella enterica typhimurium* ATCC 14028 and *Vibrio cholera* ATCC 9027. All microorganisms were stocked in appropriate conditions and regenerated before used.

### 2.4. Determination of MIC

The MIC was examined by broth dilution method in nutrient broth using a method described previously (Al-Reza et al., 2010) with a modification. Briefly, active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in nutrient broth (NB) medium and incubated at 37 °C for 24 h. The cultures were diluted with NB broth to achieve an optical density of  $10^7$  CFU/mL for the test organisms at the wavelengths of 600 nm by UV/Vis Spectrophotometer. Essential oils were diluted to get the final concentration ranging from 0 to 1000  $\mu\text{g/mL}$  in NB medium. Finally, 20  $\mu\text{L}$  inoculums of each bacteria strain was inoculated and the tests were performed at a final volume of 5 mL. The plates were incubated at 37 °C for 24 h. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in  $\mu\text{g/mL}$ .

### 2.5. Analysis of volatile chemical constituents

The volatile constituents of n-hexane extract of the plants were analyzed using GC-MS system (Agilent

6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ). The carrier gas was helium at flow rate of 1.3 ml/min, and 2  $\mu\text{L}$  of sample was injected. The electron impact technique (70eV) was used. The injector and detector temperatures were 250 °C and 230 °C.

## 3. Results and Discussion

MIC of crude extracts of spices are shown in table 1. Crude extracts of *O. x citriodorum*, *C. citratus*, *S. polyanthum* and *S. aromaticum* inhibited the growth of *B. subtilis* with MIC 31.25, 31.25, 125 and 62.5  $\mu\text{g/mL}$ , respectively. *S. polyanthum* crude extract showed a stronger inhibitory activity against *S. enterica typhimurium* (MIC = 31.25  $\mu\text{g/mL}$ ) than those of *S. aromaticum*, *O. x citriodorum* and *C. citratus*. The growth of *S. aureus*, *E. coli* and *V. cholera* were not inhibited by all crude extracts.

The polarity of solvent used in extraction of the plants plays an important role. They determine the active compounds extracted and their bioactivity. The previous report showed that MIC of n-hexane extract of *O. x citriodorum* against *S. aureus* and *S. typhimurium* were much higher than our study, they were more than 10000  $\mu\text{g/mL}$  (Ekwenchi et al., 2014).

The previous data of MIC of methanolic and ethanolic extracts of *C. citratus* against *E. coli*, *Salmonella* sp., *S. aureus*, and *B. subtilis* were much higher compare to the result of our study (Fagbemi et al., 2009; Ushimaru et al., 2007). Chloroform extract of *C. citratus* possessed the strongest antimicrobial activity, its MIC against *E. coli*, *S. aureus* and *S. typhi* were 20.0, 24.0 and 14.0  $\mu\text{g/mL}$ , respectively (Ewansiha et al., 2012), and is lower than our data.

The MIC of n-hexane extract of *S. aromaticum* against *B. subtilis* is considerably lower than data reported previously (Pundir et al., 2010). Our data is consistent with previously reported MIC of methanolic and ethanolic extracts of *S. aromaticum* against *S. aureus*, *S. typhimurium* and *E. coli*, they were higher than 1000  $\mu\text{g/mL}$  (Khan et al., 2009; Pandey and Singh, 2011; Ushimaru et al., 2007).

Extract of *S. polyanthum* is active against *B. subtilis* and *S. typhimurium* with a relatively low MIC, they are 62.5 and 31.25  $\mu\text{g/mL}$ . To the best of our knowledge, this is the first report on the MIC of n-hexane extract of *S. polyanthum*.

The antimicrobial activity of spices is usually correlated with their essential oils. Some constituents of essential oils are nonpolar, so they can be extracted

Table 2. The major volatile compounds of n-hexane extract of the spices

No	Compound Name	RT (min)	Percentage (%)			
			<i>O. x citriodorum</i>	<i>C. citratus</i>	<i>S. aromaticum</i>	<i>S. polyanthum</i>
1	$\alpha$ -methyltoluene	4.411	-	14.679	-	-
2	o-dimethylbenzene	4.602	-	11.249	-	-
3	citral B	23.325	16.022	-	-	-
4	citral A	25.319	23.696	-	-	-
5	p-eugenol	30.115	-	-	55.607	-
6	$\beta$ -caryophyllene	32.179	-	-	12.799	-
7	squalene	59.759	4.525	-	3.075	30.445
8	1,19-eicosadiene	62.482	-	12.972	-	-
9	n-hexatriacontane	63.297	-	-	-	6.573
10	n-octacosane	63.343	8.275	-	-	-
11	n-triacontane	64.703	13.448	-	8.674	0.940
12	germacrene D-4-ol	64.813	-	16.277	-	-
13	octadecyl aldehyde	65.340	-	10.473	-	-

with n-hexane (Schmidt, 2010). In our study, we conducted GC-MS analysis to determine the volatile compounds in n-hexane extract of *O. x citriodorum*, *C. citratus*, *S. aromaticum* and *S. polyanthum*. The major volatile compounds of the spices are shown at table 2.

The antimicrobial activity of crude extract of spices is related to their chemical constituents. It has been reported that essential oils containing aldehydes or phenols showed the highest antibacterial activity, followed by essential oils containing terpene alcohols. Other essential oils, containing ketones or esters, acetate had much weaker activity. While volatile oils containing terpene hydrocarbons were usually inactive (Bassolé and Juliani, 2012). Citral A and B are aldehyde monoterpenes and responsible for antimicrobial activity of *O. x citriodorum* extract against *B. subtilis* and *S. typhimurium*. Both compounds have been reported possessing antimicrobial activity (Belletti et al., 2010; Sato et al., 2006; Somolinos et al., 2009).

The volatile compounds of *C. citratus* that might be responsible for its antimicrobial activity are germacrene D-4-ol (a terpene alcohol) and octadecyl aldehyde. p-eugenol is the compound responsible for antimicrobial activity of *S. aromaticum* extract against *B. subtilis*. p-eugenol has been reported widely as a antimicrobial compound against various microorganisms (Ali et al., 2005; Leite et al., 2007; Michiels et al., 2007; Oyedemi et al., 2009; Tippyatum and Chonhenchob, 2007).

*S. polyanthum* possesses the best antimicrobial activity among four spices. Its major volatile compounds were squalene (30.445%) and n-hexatriacontane (6.573%), both are hydrocarbons considered inactive as antimicrobial. Hence, the compound responsible for antimicrobial activity might be not volatile compound and therefore was not detected by GC-MS.

#### 4. Conclusion

The antimicrobial activity of n-hexane extracts of *O. x citriodorum*, *C. citratus*, *S. aromaticum* and *S. polyanthum* against food borne *B. subtilis* and *S.*

*typhimurium* suggest that spices have potential use as food preservative.

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