Inhibition of Listeria monocytogenes by natural antimicrobial

by Silvia Oktavia

Inhibition of *Listeria monocytogenes* by natural antimicrobial

T Budiati*[1], Y Wibisono[1], R A Pambayun[2], M F Fahrezy[2], R Ariyani[2], E Kurniawati[1], W Suryaningsih[1], S O N Yudiastuti[1] and A Bakri[2]

1 Food Engineering Department, Politeknik Negeri Jember, Mastrip PO Box 164 Jember 68101 East Java, Indonesia
2 Food Industrial Technology Department, Politeknik Negeri Jember, Mastrip PO Box 164 Jember 68101 East Java, Indonesia

Email : titik_budiati@polije.ac.id

Abstract. The aim of the study was to evaluate antimicrobial activity of essential oil from plants as natural antimicrobial to inhibit *Listeria monocytogenes*. A total of 6 essential oil extracted from galangal (*Alpinia galanga*), ginger (*Zingiber officinale*), lemongrass (*Cymbopogon citratus*), orange skin (*Citrus sinensis*), rosemary (*Rosmarinus officinalis*) and turmeric (*Curcuma longa*). By using Minimum Inhibitory Concentration (MIC), the highest and lowest antimicrobial activity of essential oil to inhibit the growth of *L. monocytogenes* was found on lemongrass oil (0.32 % ± 0.12 %) and turmeric (7.46 % ± 2.79%), respectively. By using disc diffusion assay, the highest antimicrobial activity to against *L. monocytogenes* was shown by lemongrass oil (7.46 ± 2.79 mm). There is no antimicrobial activity observed in orange peel oil. Out of the essential oil tested, lemongrass oil showed the most promising natural antimicrobial to inhibit *L. monocytogenes.*

1. Introduction

Essential oils, the natural antimicrobial, have been known for decades and, with increasing demand from changes in food consumer trends and isolation of antibiotic resistant microorganisms, being other to chemical based antimicrobial which introduce the accumulative residue and give an adverse human health. Essential oil are composed by bioactive compounds which known as generally recognized as safe (GRAS) to food and might be required by food industries for ensuring food safety. Xie *et al.* [1] revealed that the biological agents of essential oils including phenolics and polypenols have to be high activity against pathogenic bacteria [1,2].

L. monocytogenes is a Gram-positive bacteria and become the main causal agent of listeriosis [3,4], the rare but lethal food-borne disease, especially for susceptible group [5]. This group was categorized as young, old, pregnant, and immunocompromised (YOPI) [6]. The contamination of *L. monocytogenes* may occur during food chain and could be spread to food processing equipments and may contaminate food products [7,8].

The study of essential oils, natural antimicrobials, extracted from Indonesia plants has to be investigated further. To do so, this study was aiming to evaluate antimicrobial activity of essential oil from plants as natural antimicrobial to inhibit *Listeria monocytogenes*. Nevertheless, it becomes a need to get the effective antimicrobial and safe to human health.

2. Material and methods

2.1. Essential oil

Six essential oils namely lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), galangal (*Alpinia galanga*), orange peel (*Citrus sinensis*), rosemary (*Rosmarinus officinalis*) and turmeric (*Curcuma longa*). The pure essential oils were obtained from Happy Green (Indonesia).

2.2. The strains of microorganism

Pure culture of *L.monocytogenes* (Nusaroma, Gajah Mada University, Yogyakarta) was cultured on Agar Listeria Ottavani and Agosti (ALOA) (Himedia, India) with supplement of ALOA (Himedia, India) for 24 until 48 hours at 37°C. The colonies of *L. monocytogenes* were appeared as blue colonies with halo surround the colonies. By using streaking method, a single colony was purified onto culture media namely tryptic soya agar (TSA) (Himedia, India).

2.3 Minimum Inhibitory Concentration (MIC)

MIC was measured as done by Budiati *et al*. [2]. Cultures were grown at 37 ºC for 16 hours in Luria Broth (LB, Himedia, India) in shaking incubator. The cultures were diluted and compared the turbidity. This has to be set at 0.5 of McFarland scale. A serial concentration was prepared on Mueller Hinton Broth (Oxoid, United Kingdom). A total of 20 µL of the aliquot was artificially contaminated to the serial dilution. These were incubated at a temperature of 37 ºC for 24 hours. MIC value (%) describes the lowest concentration of the bacteria might grow. These values are calculated as mean values of three measurements. The experiment was done in triplicate. A 15 μl aliquot of 10% DMSO was dropped onto a serial dilution as negative control. Penicillin G (10 unit/ml) was used as positive control.

2.4 Disc diffusion assay

By using disc diffusion assay, the antimicrobial activity was measured as the method of Bauer [9]. Cultures were grown at 37 ºC for 16 hours in Luria Broth (LB, Himedia, India) in shaking incubator. The cultures were diluted and the turbidity was set at 0.5 of McFarland scale. By using a sterilized cotton swab, the cultures were swabbed onto Mueller Hinton Agar (Oxoid, United Kingdom) and were let about 30 min. Twenty microliter essential oil was dropped on sterilized disc. This disc was then placed onto the agar. This was incubated for 18–24 h at 37 °C. The inhibition zones was measured in four different directions after the incubation period. This was recorded as milimeter. The experiment was done in triplicate. Fifteen microliter aliquot of 10% DMSO was dropped onto a sterile paper disc as negative control. Penicillin G (10 unit/ml) was used as positive control.

2.5 Statistical Analysis

By using one way ANOVA (SPSS version 13.0), the differences of antimicrobial activity among essential oils to inhibit the growth of *L. monocytogenes* were determined at a significance level of $P < 0.05$.

3. Results and Discussion

Wide-spectrum of antimicrobial extracted from plant namely essential oils to against Gram-positive bacteria have been well documented [10,11]. *L. monocytogenes* is a Gram-positive bacteria that can be inhibited by essential oil [12,13,14]. By using disc diffusion assay (inhibition zone) and MIC, the antimicrobial activity of six different essential oils extracted from plant in Indonesia to against *L. monocytogenes* was studied. This present study found that the highest antimicrobial activity to inhibit *L. monocytogenes* was observed on lemongrass as shown in table 1. This is similar to other study that reported that lemongrass was effective to inhibit Gram-positive bacteria such as *L. monocytogenes* [15].

Table 1. Inhibition zone and Minimum Inhibitory Concentration (MIC) of food-borne pathogenic bacteria *L.monocytogenes* on six different essential oils.

Essential oil	*Inhibition zone* (mm)	MIC (%)
Lemongrass	7,46 ± 2,79 [b]	0,32 ± 0,12 [a]
Ginger	1,11 ± 0,14 [a]	1,56 ± 0 [c]
Galangal	0,67 ± 0,60 [a]	2,60 ± 0,01 [b]
Orange peel	nd	3.13 ± 0 [d]
Rosemary	1,29 ± 0,58 [a]	3.13 ± 0 [d]
Turmeric	0,58 ± 0,52 [a]	12,5 ± 0 [e]

Note : [a,b] = different alphabet means significant different at P<0.05 in the same column
nd = not detected

L. monocytogenes was composed by peptidoglycan which was disrupted due to the exposure of citrate and other terpene in lemongrass. The devastating effect in the microbial cell by disrupting the cell membrane integrity by this compound may inhibit the growth of *L. monocytogenes* [16, 17, 18]. This may disrupt to the fatty acid biosynthesis and peptidoglycan biosynthesis [19]. Hadjilouka *et al.* [19] also reported that the gene expression in *L. monocytogenes* was observed due to the exposure of lemongrass essential oil. The genes were *accP, accA* and *fapR* that involved in fatty acid biosynthesis. Moreover the expression genes of *murR* and *pbpB* was also reported to be downregulated [19] to cause the disruption of peptidoglycan biosynthesis.

The composition of lemongrass was majority composed by geranial and neral [19]. The other compounds were also reported by Hadjiloka [19]. These were camphene, limonene, γ-cadinene, geraniol, 4-nonanone, isogeranial, α-pinene, citronellal, eucalyptol, sabinene, isoneral, caryophyllene oxide, geranyl acetate, isoeugenol, 6-methyl-5-hepten-2-one, β-Pinene, myrcene, linalool, (ω)-β-ocimene, chrysanthemal, (ε)-caryophyllene, α-Pinene epoxide, δ-Cadinene, (ε)-β-Ocimene, isoborneol, α-Terpineol, decanal, geranyl formate, geranyl butyrate, rose furan oxide, trans-piperitol, (ε)-γ-Bisabolene, tricyclene, epichrocitral, β-Elemene. The compounds may introduce the downregulation of *hly* and *inlJ* [19]. Those genes played an important role for the interaction between pathogenic bacteria with the host during infectious process [20].

This present study observed that the MIC value of ginger oil, galangal oil, orange peel oil, rosemary oil and turmeric oil were significant different for each other (table 1). However, inhibition zone of ginger oil, galangal oil, orange peel oil, rosemary oil and turmeric oil were not significant different. It indicated that all those essential oil were not effective to inhibit *L. monocytogenes* compared to lemongrass oil. The lowest antimicrobial activity was observed on turmeric oil by using MIC. The majority compound of turmeric was curcurmin and other compounds such as camphor, terpenes, lactones, alkaloids, and phenols [21]. The presence of those compounds indicated to be not effective to against *L. monocytogenes*. This is similar to other studies. Thongson *et al.* [22] reported that turmeric had no antimicrobial effect against *L. monocytogenes*. There is no antimicrobial activity was observed on orange peel oil. The compound of orange peel oil was dominated by limonene [23]. In contrary, the study of Fisher and Philips [23] reported that orange peel had antimicrobial effect to *L. monocytogenes*.

4. Conclusion

Lemongrass oil showed the highest antimicrobial effect to against the growth of *L. monocytogenes*. These essential oil seem to be a potential natural antimicrobial for inhibiting *L. monocytogenes* which was safe to food product. This was also a potential natural antimicrobial that could be used as disinfectant to clean food processing equipments which was vulnerable for the growth of *L. monocytogenes* as biofilms.

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