Antibacterial effects of essential oils of *Cymbopogon citratus* and *Amomum compactum* under self-nanoemulsifying drug delivery system (SNEDDS)

T Ujilestari¹, R Martien², B Ariyadi³, N D Dono³, Zuprizal*³

¹Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia
²Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
³Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

Corresponding Author: zuprizal@ugm.ac.id

Abstract. Microbial populations in the gastrointestinal tracts of broiler chicken can be modulated by herbal additives that contained phytochemical substances. Essential oils (EOs) have potential phytochemical compounds but are lipophilic and have low solubility in water. Therefore, self-nanoemulsifying drug delivery system (SNEDDS) might be one of the formulation strategies to improve the solubility of essential oils. In this study, the antibacterial activity of *Cymbopogon citratus* and *Amomum compactum* EOs by SNEDDS was conducted using disc diffusion method on *Escherichia coli* and *Salmonella typhimurium*. Inhibition zone was found on both pure EOs and SNEDDS formulations. SNEDDS formulations of *C. citratus* and *A. compactum* EOs were effective against *E. coli* and *S. typhimurium* (P<0.05). *C. citratus* essential oil has the highest inhibition zone when compared to the other formulations, followed by *A. compactum* essential oil. On the other hand, SNEDDS formula of *C. citratus* and *A. compactum* essential oil showed similar antibacterial activity as tetracycline and penicillin antibiotics in inhibiting the growth of *E. coli* and *S. typhimurium*. It can be concluded that the use of SNEDDS formula of essential oil would be useful to reduce the population of pathogen in the intestine of poultry.

1. Introduction

Unlike antibiotics, herbal additives are available in the nature and have fewer side effects. Herbal additives have potential phytochemical compounds and can modify the gut microflora composition in broiler chickens [1,2], hence have growth-promoting effects. The use of herbs as feed additives depends on the chemical compounds, herbal characteristics, and its purposes [2]. Essential oils (EOs) – as the part or herbal additives – have antibacterial, antiviral, antifungal, antioxidant, and insecticidal activities. EOs are widely used in pharmaceutical and food industries [3]. EOs are generally extracted by the distillation method, contain volatile molecules namely terpenes and terpenoids, aliphatic compounds, and aromatic compounds derived from phenols [4].

Lemongrass (*Cymbopogon citratus*) and cardamom (*Amomum compactum*) essential oils are good candidates as a natural alternative for antimicrobial growth promoters in poultry production. The
major components of *Cymbopogon citratus* essential oil are neral 39.0%; geranial 33.3%; limonene 5.8%; and geranyl acetate 4.2% [5]. The major components of *Amomum compactum* essential oil are 1.8-cineole 59.3%; d-limonene 29%; α-pinene 4.8%; β-pinene 4.8%; and α-terpineol 0.4% [6]. Essential oils are insoluble in water, which limits their use in the application of oral administration [7]. Self-nanoemulsifying drug delivery systems (SNEDDS) were developed to increase the solubility of an active compound [8]. Essential oils will be more effectively transported to the site of infection [7].

Essential oils have antimicrobial activities and are very influential to be explored further [9]. Antibacterial properties examinations are commonly done using diffusion and dilution methods. The agar diffusion method can be used as a paper disc [10]. The objective of this study, therefore, was to investigate the antibacterial activities of EOs of lemongrass and cardamon of both SNEDDS formulation and pure essential oils in inhibiting the growth of *Escherichia coli*, *Salmonella typhimurium*, and *Lactobacillus acidophilus*.

2. Material and methods

The Gas Chromatography-Mass Spectrometry (GC-MS, QP 2010 S, Shimadzu, Kyoto, Japan) was used to determine the chemical composition of essential oils with a standard citral at 95 % purity (Sigma-Aldrich, St. Louis, MO, USA) and 1.8-cineole at 99 % purity (Sigma-Aldrich, St. Louis, MO, USA). SNEDDS of *Cymbopogon citratus* essential oil consisted of *C. citratus* essential oil (Lansida, Yogyakarta, Indonesia), Tween 80 (Kao Indonesia Chemical, Bekasi, Indonesia), PEG 400 (idCHEM Co.,Ltd., Kyunggi, South Korea), and Virgin Coconut Oil/VCO (Healthy Co, Yogyakarta, Indonesia) at 7.147, 71.417, 14.290, and 7.147 % respectively; SNEDDS of *Amomum compactum* essential oil consisted of *A. compactum* essential oil (Orizho Indonesia, Yogyakarta, Indonesia), Tween 80, PEG 400, and VCO at 10, 65.71, 14.29, and 10 % respectively.

The antibacterial properties were determined using the disc diffusion method. Penicillin 128μg/ml per disc (Penicillin-G Meiji, Pharmaceutical Industries, Pasuruan, Indonesia) and tetracycline 128μg/ml per disc (Super Tetra, Darya Varia Laboratoria, Jakarta, Indonesia) were used as a positive control. Each treatment (pure essential oils and SNEDDS essential oils) with a concentration of 20 μl was placed in a paper disc 5 mm diameter (OXOID Limited, Basingstoke, Hampshire, UK), evaluated as antibacterial agents against *Escherichia coli* FNCC 0091, *Salmonella typhimurium* FNCC 0050, and *Lactobacillus acidophilus* 0051 (Food and Nutrition Development and Research Center, Universitas Gadjah Mada, Yogyakarta, Indonesia) using Kirby-Bauer disc diffusion method. The plates were incubated at 37°C for 48 h, and then the antibacterial activity was subsequently determined by measuring the diameter of inhibition zones with calipers. The samples were replicated five times and the means ± standard deviations were reported. Results of the inhibition zone were analyzed using one-way analyses of variance (ANOVA) followed by Duncan’s new Multiple Range Test, using P-value for less than 5% for a statistically significant statement [11].

3. Results and discussion

The antibacterial activity of essential oils was probably attributed to the volatile components. Citral, the main active components found in *Cymbopogon citratus* essential oil that consists of geranial and neral, was reported to have antibacterial activities to inhibit the growth of pathogenic bacteria, such as *Salmonella typhimurium*, *Escherichia coli*, *Campylobacter jejuni*, *Clostridium perfringens*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Bacillus cereus* [12,13,14]. Meanwhile, the major constituent 1.8-cineole in *Amomum compactum* essential oil has the ability to inhibit the growth of *Salmonella typhimurium*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Methicillin-resistant Staphylococcus aureus* [15,16,17].

Table 1 showed the antibacterial potency of *Cymbopogon citratus* and *Amomum compactum* essential oil. The inhibition zones (mm) indicated that the *Cymbopogon citratus* essential oil (2μl) showed highly active against *Escherichia coli*, *Salmonella typhimurium*, and *Lactobacillus acidophilus*. The *Amomum compactum* essential oil showed a moderate effect against *Escherichia coli*, *Salmonella typhimurium*, and *Lactobacillus acidophilus*. SNEDDS of *Cymbopogon citratus* and...
Amomum compactum essential oil showed the same antibacterial activity as tetracycline and penicillin antibiotics in inhibiting the growth of Escherichia coli and Salmonella typhimurium (Gram-negative bacteria species), although the inhibition of Lactobacillus acidophilus (Gram-positive bacteria species) was higher compared to antibiotics. Essential oils from herbal plants have potential as antibacterial sources, but their activity is influenced by the test methods and bacterial strains [18].

| Treatment | Escherichia coli FNCC-0091 (mm) | Salmonella typhimurium FNCC-0050 (mm) | Lactobacillus acidophilus 0051 (mm) |
|-----------|---------------------------------|--------------------------------------|-----------------------------------|
| T1        | 2.72±0.73b                      | 6.60±2.80bc                         | 6.60±0.79b                        |
| T2        | 3.64±0.90b                      | 7.48±1.10bc                         | 6.12±0.44bc                       |
| T3        | 3.44±1.51b                      | 5.52±1.00bcd                        | 4.96±0.09ed                       |
| T4        | 2.72±1.30b                      | 4.72±2.00cd                         | 4.98±0.29ed                       |
| T5        | 20.24±9.80a                     | 24.00±1.00a                         | 9.00±2.24a                        |
| T6        | 6.04±1.10b                      | 8.44±0.80b                          | 5.26±0.30ed                       |
| T7        | 2.48±0.70b                      | 6.88±5.20bc                         | 4.66±0.27d                        |
| T8        | 4.80±2.00b                      | 6.68±3.50bc                         | 4.26±0.82d                        |
| T9        | 3.56±0.60b                      | 2.60±0.90d                          | 4.78±0.74d                        |

| Statistic | SEM  | P-value |
|-----------|------|---------|
|           | 0.926| <0.001  |
|           | 0.944| <0.001  |
|           | 0.241| <0.001  |

1Data represent means from five replicates plate.
2Means within a column without a common superscript differ significantly (P<0.05).
3T1: SNEDDS of Cymbopogon citratus essential oil; T2: SNEDDS of Amomum compactum essential oil; T3: SNEDDS blank of Cymbopogon citratus essential oil; T4: SNEDDS blank of Amomum compactum essential oil; T5: Cymbopogon citratus essential oil; T6: Amomum compactum essential oil; T7: Penicillin (128 μg/ml); T8: Tetracycline (128 μg/ml); T9: VCO.

Analysis using GC-MS indicated that in Cymbopogon citratus essential oil, α-citral (38.04%) and β-citral (29.31%) were the main components, whereas 1.8-cineole (63.80%) was the main component in Amomum compactum. The major components of Cymbopogon citratus essential oil were citral (aldehydes geranial 40-62% + neral 25-38%), and the other main components were terpenes [19]. The major component of Amomum compactum essential oil was 1.8-cineole 59.3%; d-limonene 29%; α-pinene 4.8%; β-pinene 4.8%; and α-terpineol 0.4% [6]. The antibacterial activity of Cymbopogon citratus essential oil was due to the relatively high concentration of citral [20].

Essential oils are hydrophobic, which allows them to partition the lipids of cell membranes and bacterial mitochondria. The mechanism action of the components of essential oils is to break the bacterial cell wall, damage the cytoplasmic membrane and the membrane proteins, causing damage to cell contents, hence cytoplasmic coagulation, and depletion of proton motive force [21,22]. Some studies have found that Gram-positive bacteria to be more sensitive to essential oils than Gram-negative bacteria. This is indicated by the relatively impermeable cell walls in which the outer membrane is rich in lipopolysaccharide in Gram-negative bacteria [23]. On the other hand, the cell walls of Gram-positive bacteria are rich in unsaturated fatty acids, when the cell walls are rearranged it will result in loss of cell viability and ultimately lead to death [24]. However, the antibacterial activity of essential oils depends on the composition and volatile components of each essential oil [25].

4. Conclusion
In conclusion, this study confirmed the antimicrobial activity of active components in Cymbopogon citratus and Amomum compactum against Escherichia coli, Salmonella typhimurium, and
Lactobacillus acidophilus. SNEDDS formulation of essential oil would be useful to reduce the population of the microbial pathogen in the intestine of poultry as antimicrobial growth promoters.

References
[1] Vidanarachchi J K, Mikkelsen L L, Sims I, Iji P A and Choct M 2005 Recent Adv. Anim. Nutr. Aust. 15 131–44
[2] Hashemi S R and Davoodi H 2011 Vet. Res. Commun. 35 169–80
[3] Henri I, Bassolé N and Juliani H R 2012 Molecules 17 3989–4006
[4] Bakkali F and Idaomar M 2008 Food and Chemical Toxicology 46 446–75
[5] Vazirian M, Kashani S T and Shams M R 2012 J. Essent. Oil Res. 24 579–82
[6] Huang Y-B, Fang J-Y, Hung C-H, Wu P-C and Tsai Y-H 1999 Biol Pharm Bull. 22 642–6
[7] Natrajan D, Srinivasan S, Sundar K and Ravindran A 2015 J. Food Drug Anal. 23 560–8
[8] Date A A and Nagaresker M S 2007 Int. J. Pharm. 329 166–72
[9] Millezi A F, Caixeta D S, Rossoni D F, Cardoso G and Piccoli R H 2012 Ciênc. Tecnol. Aliment. 32 167–72
[10] Kalemba D and Kunicka A 1989 J. Essent. Oil Res. 1 119–28
[11] Abbassia F, Boujdjella H, Zitouni A and Hassani A 2014 ExCLI J. 13 772–81
[12] Wannissorn B, Jarkasem S, Siriwanjai T and Thubthimthed S 2005 Fitoterapia 76 233–6
[13] Vyshali P, Suchetha M and Saraswathi K J T 2016 Journal of Agricultural Science 1 35–41
[14] Zulfa Z, Chia C T and Rukayadi Y 2016 Int. Food Res. J. 23 1262–7
[15] Nanosombat S and Lohasupthawee P 2005 KMITL Sci. Technol. J. 5 527–38
[16] Zengin H and Baysal A H 2014 Molecules 19 17773–98
[17] Jamil B, Abbasi R, Abbasi S, Imran M, Khan S U, Ihsan A, Javed S, Bokhari H and Imran M 2016 Front. Microbiol. 7 1–10
[18] Bagamboula C F, Uyttendaele M and Debevere J 2004 Food Microbiol. 21 33–42
[19] R.R.B N 2007 Rev. Bras. Pl. Med. Botucatu 9 80–92
[20] Citratus C and Staaf D C 1984 J. Ethnopharmacol. 12 279–86
[21] Gracia-Valenzuela M H, Orozco-Medina C and Molina-Maldonado C 2012 Hidrobiologica 22 201–6
[22] Li S 2011 Enhancement of the antimicrobial activity of eugenol and carvacrol against Escherichia coli O157: H7 by lecithin in microbiological media and food Thesis (Tennessee: University of Tennessee)
[23] Nakaido H 1994 J. Biol. Chem. 269 3905–8
[24] Claeson P, Rhdstrom P and Skold O 1992 Phyther. Res. 6 94–8
[25] Dorman H and Deans S 2000 J. Appl. Microbiol. 88 308–16