

RESEARCH ARTICLE

Comparison of Antimicrobial Activity between Ethanolic Extract and Essential Oil of Ginger (*Zingiber officinale*) against Food-borne Bacteria

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ABSTRACT

Background: Ginger (*Zingiber officinale*) is one of the most well-known spices with antimicrobial activity. However, different extraction methods of ginger will result in different antimicrobial properties due to the various substances extracted. This study aimed to compare antimicrobial activity between ethanolic extract and essential oil of ginger against food-borne bacteria grown in 2.1% Mueller Hinton agar. **Methods:** Fresh ginger rhizomes were extracted either by using vapor distillation method or maceration using ethanol to obtain ginger essential oils (GEO) and ginger ethanolic extract (GEE), respectively. Ethanolic extract and essential oil of ginger at different concentrations were then tested for their antimicrobial activity using disk diffusion method against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus*. Tetracycline was also used as a standard of antibacterial agent. **Results:** Ginger essential oil in 25%, 50%, and 100% showed significant growth inhibition of four types of bacteria compared to ginger ethanolic extract. This antimicrobial effect of ginger essential oil was shown to be dose-dependent. However, it has been demonstrated that ginger ethanolic extract 50% and 100% has a stronger antimicrobial effect against *B.subtilis*. **Conclusion:** Ginger essential oil and ethanolic extract showed different degree of antibacterial activity against food-borne bacteria due to compound contained within respective extracts, with a higher degree of activity found in ginger essential oil. These may show that different ginger extract may have different antibacterial activity.

Keywords: *Zingiber officinale*; antimicrobial activity; ginger essential oil; ginger ethanolic extract; food-borne bacteria

INTRODUCTION

Ginger (*Zingiber officinale*) is one of the most well-known spices from Indonesia. It is often used in traditional medicine in Asia (i.e., Traditional Chinese Medicine, Ayurvedic medicine, etc.) as a treatment for headaches, nausea, rheumatism and colds (Grzanna, Lindmark, Frondoza, 2005). Even though it is often used in simplicia form, the essential oils and extracts are rarely found in medicine. Nowadays, the extract or simplicia of ginger is often usually in combination with other natural products in a various pharmaceutical product called "Jamu", "Obat Herbal Terstandar", and "Fitofarmaka" for its immune-enhancing effect (Habsah et al., 2000). In addition to boosting the immune system, ginger also has other beneficial effects such as reducing risk of atherosclerosis (Azu et al., 2007) and antimicrobial activity against various pathogenic and foodborne bacteria (Habsah et al., 2000).

Food-borne bacteria is one of the leading causes of food-borne diseases such as diarrhea, typhoid, and salmonellosis (Havelaar et al., 2015). According to the WHO report in 2015, the diarrheal causing agents caused 230,000 of the 420,000 deaths globally, with shared about 54% of the total burden (WHO, 2015). Meanwhile, in Indonesia, the rate of diarrhea is 1.23% in 2015 (Kementerian Kesehatan RI, 2016). The diarrheal fatality rate of 2.47% also turned it into one of the diseases that cannot be neglected (Kementerian Kesehatan RI, 2016). This condition highlights the importance of food safety, which can be achieved by implementing good sanitation and hygiene, as well as using an antibacterial agent.

Study regarding the antimicrobial effect of ginger was already done by Hammer (1999) by using ginger essential oil towards *E. coli*, *S. enterica*, *E. faecalis*, *A. baumannii*, etc. Meanwhile, Grace, Sankari and Gopi (2017)

studied the effect of ginger ethanolic extract towards *E. faecalis* and *S. aureus* and found that the extract was more effective towards *S. aureus*. Although both data obtained from those studies are valuable, there was yet no study that compares the difference of extraction methods towards the antimicrobial activity of ginger. This information is undoubtedly required to determine which ginger extract needs to be applied in natural product, especially natural cosmetics, supplements, or even as a food preservative.

This study aims to compare how different ginger extraction method may alter the potency of its antimicrobial activity. The result may give an insight in consideration of using ginger extract as an antimicrobial agent to combat food-borne diseases. In this study, essential oil and ethanolic extract of ginger were tested against four types of food-borne bacteria; *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus*.

MATERIAL AND METHODS

Materials

Ginger rhizomes were bought from a traditional market in Jakarta. Ethanol was obtained from Merck (Germany). Polysorbate 80 (Tween 80) and Mueller-Hinton Broth were purchased from Merck (Germany). Tetracycline was purchased from Bio Basic (Canada). *E. coli* (NEB[®] catalog No. C2989K), *B. subtilis* (ATCC 6633), *S. typhi* (ATCC 14028) and *S. aureus* (InaCC B4) were generously provided by Indonesia International Institute for Life Sciences, Indonesia. Whatman paper no. 1 was obtained from Whatman (Sigma-Aldrich). Bacteriological agar was obtained from Becon (UK).

Methods

Preparation of Ginger Essential Oil - Fresh ginger was washed, cut into thin pieces and loaded into vapor distillatory and extracted for 5 hours. The essential oil of ginger was then collected into 1.5 mL amber vial and kept at refrigerator to preserve the essential oil from external damages. The testing samples were then prepared by dissolving the essential oil in 5% polysorbate 80 to obtain a concentration of 25%, 50%, and 100%.

Preparation of Ginger Ethanolic Extract - The preparation of ginger ethanolic extract was done following previously reported research (Azu et al., 2007) with some modification. Fresh ginger was washed and cut into thin pieces, and 50 g of them was transferred into an Erlenmeyer flask filled up with 200 mL of ethanol. The flask was then placed onto a shaker and shaken at 150 rpm for three days. The extract was then filtered using Whatman paper no. 1, and the residue was again added with ethanol for another extraction. Combined ethanolic extract further concentrated by using a rotary evaporator before transferred into falcon tubes covered with aluminium foil and kept inside a refrigerator for further use. The testing samples were prepared by dissolving the ginger ethanolic extract with 5% polysorbate 80 to get the concentration of 25%, 50%, and 100%.

Antimicrobial standard preparation - The standard antimicrobial used in this study was tetracycline. Tetracycline stock solution was prepared in distilled water at a concentration of 10 mg/ml. The working concentration of tetracycline was 10 µg/ml and prepared by diluting the stock solution with distilled water.

Disk Diffusion method - Antibiotic activity of ginger essential oil and ginger ethanolic extract was assessed by using disk diffusion

method (De Silva et al., 2017). The media used was 2.1% Mueller-Hinton broth mixed with 1.4% bacteriological agar in distilled water, sterilized and poured into Petri dishes. Bacteria stock solutions were prepared at a concentration equal to 0.5 McFarland units (1.5×10^8 CFU/mL) before then inoculated onto the media. Prepared sterile disks made from Whatman filter paper no. 1 with a diameter of 5 mm were dipped into each working solution of samples and standard 1 hour before testing. Thereafter, the disk was placed on top of the media that have been inoculated with bacteria in triplicate manner. Solvent control was also prepared by testing the effect of 5% polysorbate 80. All of the Petri dishes were kept for 24 hours at 37 °C. After 24 hours, the media were observed for the area of inhibition and diameter of the area is measured.

Statistical analysis - The data obtained were statistically analyzed by two-tail student t-test. The P value of 0.05 was used to determine the significance level.

RESULTS AND DISCUSSION

In this study, the antimicrobial effect of ginger essential oil (GEO) and ginger ethanolic extract (GEE) was evaluated using disk diffusion method against four types of food-borne bacteria, which were *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus*. Figure 1 shows the mean diameter of inhibition of GEO and GEE towards *E. coli*. It was observed that GEE had no antibacterial activities against *E. coli*, while GEO at a concentration of 25% showed a comparable bacterial growth inhibition with standard tetracycline. The data presented in each figure was expressed as a mean of three replications ± standard deviations.

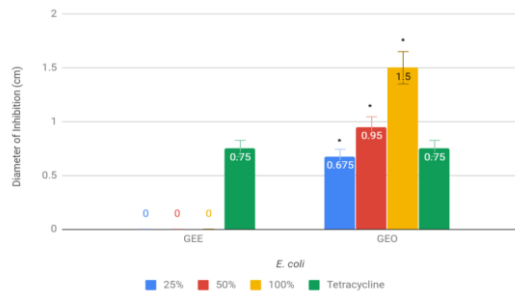


Figure 1. Mean of diameter of inhibition of ginger essential oil (GEO) and ginger ethanolic extract (GEE) towards *E. coli*. *indicated the significant differences between GEE and GEO

The antibacterial activity of GEE and GEO towards *S.typhi* and *S. aureus* were shown in Figure 2 and Figure 3, respectively. Again, GEE has not exhibited any antibacterial effect even at the highest concentration used for *S.typhi* and *S. aureus*, while GEO at 25% elicited higher antibacterial activity compared to the standard tetracycline.

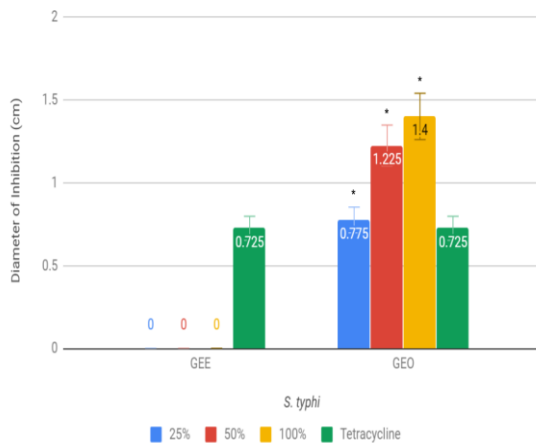


Figure 2. Mean of diameter of inhibition of ginger essential oil (GEO) and ginger ethanolic extract (GEE) towards *S. typhi*. *indicated the significant differences between GEE and GEO

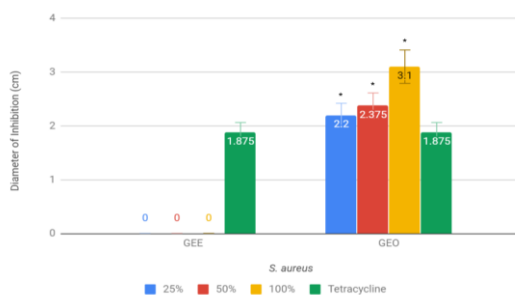


Figure 3. Mean of diameter of inhibition of ginger essential oil (GEO) and ginger ethanolic extract (GEE) towards *S. aureus*. *indicated the significant differences between GEE and GEO

The antibacterial activity of GEE and GEO towards *B. subtilis* was shown in Figure 4. It was presented that GEE at a concentration of 50% showed weak antibacterial activity against *B. subtilis* compared than GEO, although both GEE and GEO antibacterial effects were found to be inferior compared to standard tetracycline.

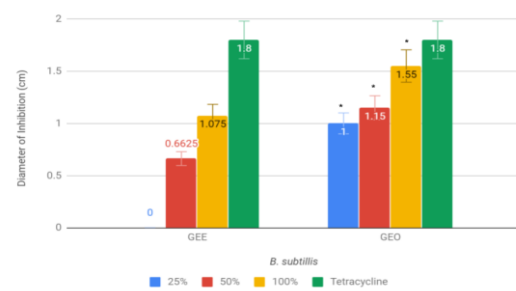


Figure 4. Mean of diameter of inhibition of ginger essential oil (GEO) and ginger ethanolic extract (GEE) towards *B. subtilis*. *indicated the significant differences between GEE and GEO

As shown in the data presented above, GEO exhibited antimicrobial activity in a dose-dependent manner, as the diameter of inhibition increased along with the concentration. On the other hand, GEE did not display any antibacterial activity against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, as shown in Figure 1, 2, and 3. Nevertheless, it showed weak antimicrobial activity against *Bacillus subtilis* at a concentration of 50% and 100%. This result might be attributed to difference compounds extracted as a result of two different extraction methods employed.

In addition, when compared to the standard tetracycline, GEO at a concentration of 50% and 100% showed higher inhibition effect towards *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*. Meanwhile, 5% polysorbate 80 used as a solvent control

did not yield any antimicrobial effect. This concluded that the bacterial growth inhibition properties only came from the testing material.

Ginger was reported to contain several active ingredients, including terpenes and oleoresin (Rahmani, Shabrmi, Aly, 2014). The terpenes can be further identified into sesquiterpene hydrocarbons and phenolic compounds such as gingerol and shogaol. Lipophilic extracts of ginger mostly contain active gingerols which then can be converted into shogaols, zingerone, and paradol (Rahmani, Shabrmi, Aly, 2014). Another study done by Park, Bae and Lee (2008) stated that the antimicrobial activity of ginger was significantly attributed to the presence of gingerol. The antimicrobial efficacy of gingerol was also influenced by variation in the length and modification of alkyl side chain. It was observed that [10]-gingerol exhibited antibacterial effect and growth of *Helicobacter pylori*. [12]-gingerol has also shown to cause growth inhibition of some pathogenic bacteria with MIC values dependant on the alkyl side chain length (shorter alkyl chain of gingerol yields higher MIC) (Hiserodt, Franzblau, Rosen, 1998). Furthermore, the presence of the acetoxy group on the gingerol resulted in loss activity of antibacterial (Park, Bae, Lee, 2008).

Previously, it was reported that ginger ethanolic extract showed higher antimicrobial activity against gram-positive bacteria as tested for *S. aureus* and *E. faecalis*, respectively (Grace, Sankari, Gopi, 2017). This is somewhat contradicted our result, which showed that GEO inhibited the growth of gram-positive *B.subtilis* rather than the other gram-negative bacteria. This disparity might be attributed to the different geographical origin of the ginger used in these experiments. A study done by Salmon et al. (2012) reported that different geographical regions of ginger in Jamaica significantly

varied the content and the total pungency of ginger. Hence, it could be assumed that the discrepancy found in this study was due to the different origin of ginger.

Both GEO and GEE were predicted to contain gingerol due to the pungent characteristic smell that was generated. However, the concentration of gingerol contained in each extract might be varied, which contributed to the significant differences observed in the result. It was predicted that the GEE might contain different type of gingerol (longer alkyl chain substituted gingerol) or contain gingerol with acetoxy modification, which caused a lack of antimicrobial activity in this study.

In future studies, we recommend to further purify and analyze phytochemical compounds contained in each type of extracts using Nuclear Magnetic Resonance (NMR) or mass spectroscopy (MS). Furthermore, each phytochemical could be quantized and checked for another antimicrobial activity.

CONCLUSION

Ginger essential oil and ginger ethanolic extract showed different degree of antibacterial activity against four types of food-borne bacteria (i.e., *E. coli*, *B. subtilis*, *S. typhi*, and *S. aureus*), with higher effectivity overall being found in GEO compared to the GEE. It was predicted that the differences came from different types of gingerol obtained during the extraction process. It is recommended to identify the phytochemical contents inside each extract in correlation to the disparity in the results. Nevertheless, this result could provide an insight to consider the type of ginger extract to be harnessed as an antibacterial agent to combat food-borne diseases.

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