In vitro antibacterial activities of Syzygium polyanthum leaves extract-nanoparticle against Salmonella typhimurium, Escherichia coli, and Lactobacillus acidophilus

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Abstract. A research with six treatments in a completely randomized design was conducted to evaluate the antibacterial activities of Syzygium polyanthum leaves extract (SpLE)-nanoparticles against Salmonella typhimurium FNCC-0050, Escherichia coli FNCC-0091, and Lactobacillus acidophilus FNCC-0051. The disc diffusion method was used to measure antibacterial activities of the bay leaves extract. The sample plates were incubated at room temperature 37 °C for 24 hours, and the antibacterial activity was further determined by measuring the inhibition zone diameter manually using a precision ruler. The treatments were: aquades only (SpLE-0), aquades with 50 ppm antibiotic Tetracycline (SpLE-1), aquades with 0.2% chitosan (SpLE-2), aquades with 0.04% Sodium triplosphate (SpLE-3), aquades with 2.0% SpLE (SpLE-4), and aquades with 2.0% SpLE-N (SpLE-5). One-way ANOVA was used to analyse data statistically and if there were significant differences, it was followed by Duncan's new multiple range test. The results showed that there was an inhibition zone in SpLE against S. typhimurium, E. coli, and L. acidophilus (P<0.001). SpLE-N and chitosan inhibited S. typhimurium and E. coli (P<0.001). While Tetracycline inhibited only E. coli (P<0.001). Currently, findings show that S. polyanthum leaf extract was able to inhibit the growth and colonization of several pathogenic bacteria.

1. Introduction

Started from 3 years back, the Government of Indonesia has decided to ban the addition of antibiotics in the diet of poultry through MOA 14, 2017, Article 16 concerning Classification of Veterinary Drugs. The decision was made due to the reports that the presence of antibiotics in the diet produced residues in livestock products that could be harmful to human health [1,2]. Prohibition against the use of antibiotics has caused the emergence of old diseases, such as: necrotic enteritis, that affect in economic losses to farmers. Therefore, scientists are trying to find alternatives to antibiotics with other types of feed additives. Current feed additives that are shown to have the likely capability as alternatives for antibiotic growth promoters are: photobiotic (herbal feed additives), probiotics, prebiotics, symbiotic, organic acids and others [3]. Phytobiotics are natural compounds derived from plants which are expected to increase livestock productivity [4].

One alternate plant that can be used as phytobiotic is bay (Syzygium polyanthum) leaves. As a traditional medicine, bay leaves have been proven to have many pharmacological functions, such as antioxidant, antihypertensive, immunomodulatory, anticancer, and antidiarrheal [5-7]. The beneficial properties of S. polyanthum could be attributed to the content of numerous secondary metabolite
compounds in the leaves [8], such as: phenolic, saponins, flavonoids and alkaloids which have activities such as antibacterial, antioxidant, and so on [9]. Extracts of the S. polyanthum leaves have been reported to show anti-cholesterol, anti-fungal, antibacterial, and anti-inflammatory properties [10-13].

Nano encapsulation, a developed technology to capsule or coat bioactive substances in nano dimension, was declared to be beneficial to improve thermal stability, oral bioavailability, and water solubility [14,15] of phytochemicals. Nano encapsulation potential to released periodically the active ingredients through the encapsulation layer, thereby increasing the efficiency of using the active ingredients for the host animal [16]. The release of bioactive compounds can be controlled with Nano encapsulation system so it can be distributed at the right time and place [17]. Through in vitro - diffusion method, the antibacterial activities of the plant-bioactive compounds can be examined prior application in livestock. Therefore, this study aims to determine the antibacterial activity of SpLE-nanoparticles in inhibiting the growth of pathogenic bacteria E. coli, S.typhimurium, and L. acidophilus.

2. Materials and methods

2.1. Materials

Syzygium polyanthum leaves (Yogyakarta, Indonesia); Chitosan (MW, 70 kDa; deacetylation degree, 85%) (Merck, Darmstadt, Germany) and Trypolyphospathe (Bratachem, Indonesia) were used for the preparation of nanoparticles. E. coli FNCC-0091, S. Typhimurium FNCC-0050, and L. acidophilus FNCC-0051 which bought on the Food and Nutrition Development and Research Centre (FANDARC), Universitas Gadjah Mada, were used for evaluating the antibacterial activity in vitro. Nutrient agar (Difco Laboratories, Detroit, Michigan, USA) were used as growth media of bacteria.

2.2. Methods

Nanoparticles- Syzygium polyanthum leaf extract, its antibacterial activity was observed using the well diffusion method against three bacteria, namely; E. coli, S. typhimurium, and L. acidophilus. Pure microbial culture rejuvenated in MH broth (35°C). Sterile cotton is used to plant bacterial strain on each plate, and then a gel stick is used to make a 6 mm well. Samples of distilled water, tetracyclyc, chitosan, STPP, SpLE, and SpLE-nanoparticles were poured into the well using a micropipette. Petri dishes were stored in an incubator at 35 °C for 24 hours, and then a ruler was used to measure the diameter of the inhibition zone or clear zone around the well. The sample was replicated three times. The measurement of bacterial inhibition requires media for bacterial suspension culture. The medium used was broth agar by observing the inhibition using the well diffusion method. The zone inhibition formed is a clear area around the treatment and there is no bacterial colony growth or contamination [18]. The antibacterial activity of SpLE-nanoparticles needs to be compared with the packaging components (SpLE, chitosan, STPP and aquades) and antibiotic to determine the inhibitory activity of each material. One-way ANOVA is used to analysese the value of the zone of inhibition and if there is a significant value then it is tested further using the Duncan’s new Multiple Range Test [19].

3. Results and discussion

The antibacterial activities of SpLE-nanoparticle, SpLE, chitosan, STPP, distilled water, and antibiotics Tetracycline were shown in Table 1.

Table 1 show that SpLE inhibited all types of examined bacteria, while aquades and STPP did not affect all types of bacteria. The longest diameter of bacterial inhibition zone against E. coli FNCC-0091 was detected on 0.2% chitosan (P <0.001). Further inhibitory activity was found on SpLE-nanoparticle, Tetracycline, and SpLE. The longest diameter of bacterial inhibition zone against S. typhimurium FNCC-0050 was exposed on 0.2% chitosan, followed by SpLE-N (P <0.001). Further inhibitory activity was found on both SpLE- nanoparticles and SpLE. The inhibitory activity against L. acidophilus FNCC-0051 was only found on SpLE. The antibacterial activity of SpLE can be
associated with phytochemical contents, such as: total phenols, flavonoids, tannins, and alkaloids. The synergistic effect among secondary metabolites causes pharmacological effects [20,21]. The inhibition of SpLE bacteria against three types of bacteria showed the highest activity for \textit{L. acidophilus}, followed by \textit{S. typhimurium} and \textit{E. coli}.

**Table 1.** An antibacterial activity of \textit{Syzygium polyanthum} leaves extract- nanoparticles.

| Treatments                      | \textit{Escherichia coli} (FNCC-0091) | \textit{Salmonella typhimurium} (FNCC-0050) | \textit{Lactobacillus acidophilus} (FNCC-0051) |
|---------------------------------|--------------------------------------|---------------------------------------------|-----------------------------------------------|
| Aquades                         | 0.00±0.00<sup>a</sup>                | 0.00±0.00<sup>c</sup>                       | 0.00±0.00                                     |
| Tetracycline                    | 8.56±1.04<sup>b</sup>                | 0.00±0.00<sup>c</sup>                       | 0.00±0.00                                     |
| Chitosan                        | 11.22±0.67<sup>a</sup>               | 8.94±0.85<sup>a</sup>                       | 0.00±0.00                                     |
| STPP                            | 0.00±0.00<sup>d</sup>                | 0.00±0.00<sup>c</sup>                       | 0.00±0.00                                     |
| SpLE                            | 4.28±0.91<sup>c</sup>                | 4.33±0.50<sup>b</sup>                       | 5.44±0.77                                     |
| SpLE-N                          | 9.22±0.62<sup>b</sup>                | 8.78±0.51<sup>a</sup>                       | 0.00±0.00                                     |

**Statistic**

| SEM               | 1.983 | 1.777 | 0.907 |
| P-value           | <0.001| <0.001| <0.001|

<sup>a,b,c,d</sup> Different superscripts within the same column show significant difference (P<0.001); <sup>1</sup>Inhibition Zone Diameter (mm) ± SD; <sup>2</sup>Tetracycline=aquadest+50 ppm Tetracycline, Chitosan=aquadest+0.2% chitosan, STPP=aquadest+0.04% Sodium triphosphate, SpLE=aquadest+2.0% SpLE, SpLE-N=aquadest+2.0% SpLE-nanoparticles.

The inhibition power of SpLE-nanoparticle against \textit{E.coli} bacteria was lower than that of chitosan, but higher than that of both SpLE and tetracycline. This is probably due to the antibacterial activity of \textit{S. polyanthum} leaves phytochemical compounds added with chitosan. The antibacterial activity of chitosan can inhibit bacterial pathogens in the form of pathogens. This activity might be influenced by the intrinsic characteristics of chitosan, such as: molecular weight and degree of deacetylation, as well as the extrinsic characteristics, such as: pH, temperature, and the presence of other influential compounds [22,23]. The inhibition capacity of SpLE-nanoparticle against \textit{S. typhimurium} was slightly lower than that of chitosan but higher than that of SpLE. The area of inhibition of SpLE-nanoparticles and chitosan, which was almost the same, was probably due to the high inhibition level on \textit{S. typhimurium} so that although the activity of bay leaf compounds was increased with chitosan, but it still had the same activity. In addition, the absence of inhibitory activity on tetracycline indicates high level on \textit{S. typhimurium}. Whereas the inhibition of SpLE-nanoparticles against \textit{L.acidophilus} was not shown, but in SpLE there was little inhibitory activity. This suggests that the antibacterial activity of bay leaf compounds affects all types of bacteria.

The presence of inhibition against bacterial growth is influenced by the content of active compounds in bay leaves including: total phenols, flavonoids, tannins, and alkaloids. Flavonoid compounds can damage cell walls, causing cell death [24]. Flavonoids can also inhibit protein formation thereby inhibiting microbial growth. In addition to flavonoids, the content of other compounds such as tannin compounds can also damage cell membranes [25]. Alkaloid compounds as antibacterial by damaging the cell walls causing cell death. [26].

**4. Conclusions**

Currently, findings show that \textit{S. polyanthum} leaf extract was able to inhibit the growth and colonization of several pathogenic bacteria.
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