Antibacterial Activity of *Sargassum* sp. Extract Encapsulated into Chitosan-Tripolyphosphate Nanoparticles

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Abstract. This study aimed to evaluate the antibacterial activity of *Sargassum* sp. Extract encapsulated into chitosan-tripolyphosphate nanoparticles. *Sargassum* sp. Extract was prepared by maceration using ethanol 96% (1:10) for 24 h. Four nanoparticles formula were synthesized with the chitosan:*Sargassum* sp. Extract:tripolyphosphate ratios of 4:0:2 (F0), 4:1:2 (F1), 4:2:2 (F2), and 4:4:2 (F3). The nanoparticles along with aquadest, zinc bacitracin (10 mg/mL), and free *Sargassum* sp. Extract (100 mg/mL) were then tested for antibacterial activity against *Escherichia coli* and *Salmonella* sp. Using agar well diffusion method. Results showed that the incorporation of *Sargassum* sp. Extract into chitosan-tripolyphosphate nanoparticles provided a significant effect on antibacterial activity ($P < 0.05$). F2 has higher *Escherichia coli* and *Salmonella* sp. Inhibition than that of F0 and F1 ($P < 0.05$) but did not differ when compared with F3. F2 has equivalent *Escherichia coli* inhibition as compared to zinc bacitracin and free *Sargassum* sp. Extract. Moreover, F2 has higher antibacterial activity against *Salmonella* sp. ($P < 0.05$) than that of zinc bacitracin and free *Sargassum* sp. Extract. It could be concluded that *Sargassum* sp. Extract encapsulated into chitosan-tripolyphosphate nanoparticles is a promising candidate as an antibiotic alternative in poultry nutrition. Nano-F2 provided the optimum antibacterial activity against *E. coli* and *Salmonella* sp. Among the tested nanoparticles formula.

Keywords: antibiotics · nanoparticles · *Sargassum* sp. · *Escherichia coli* · *Salmonella* sp

1 Introduction

Since 1950s, antibiotics have been widely used to promote growth and prevent diseases in the livestock industry [1–3]. Although this practice provides economic benefits, the overuse of antibiotics evidently contributed to the existence of resistant microbes that threatens both human and livestock [4, 5]. For that reason, many countries were then banned the use of antibiotics as growth promoting agents in the livestock sector [6, 7]. This situation demanded the search for suitable alternatives to antibiotics.

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Sargassum sp. is one type of brown seaweed which has great potency as an antibiotics alternative. The availability of Sargassum sp. is highly abundant in the Indonesian coast area [8–11]. *Sargassum* sp. Extract is rich in phenolic compounds [12, 13] which could act as natural antibacterial substances [14–16]. However, it should be underlined that phenolic compounds are highly sensitive to the acidic condition and have poor bioaccessibility [17, 18].

Chitosan-sodium tripolyphosphate nanoparticle is one of the promising nanocarrier to overcome the limitation of phenolic compounds [19]. The incorporation of phenolic compounds into chitosan-sodium tripolyphosphate nanoparticle allowing these compounds to be remained stable under acidic condition, as well as providing better bioaccessibility and bioactivity [20–23]. Therefore, this study was aimed to evaluate the antibacterial activity of *Sargassum* sp. Extract encapsulated into chitosan-tripolyphosphate nanoparticles.

2 Materials and Methods

2.1 Materials

The materials used in this study were *Sargassum* sp. (UD. Rumput Laut Mandiri, Gunung Kidul, Indonesia), ethanol 96%, distilled water (CV. Makmur Sejati, Malang, Indonesia), glacial acetic acid (PT. Smart Lab Indonesia, South Tangerang, Indonesia), chitosan powder (CV. ChiMultiguna, Indramayu, Indonesia), sodium tripolyphosphate (Prima Rasa, Malang, Indonesia). All chemicals were used as received without any further purification.

2.2 Encapsulation of *Sargassum* sp. Extract

A hundred grams of *Sargassum* sp. Powder was macerated in 1,000 mL of ethanol 96% for 24 h at 25 °C. The extracts were then filtered through four layer of muslin cloth. Solvent was then evaporated in the water bath at 40 °C until resulting thick extract. Four nanoparticles formula were then synthesized with the chitosan:*Sargassum* sp. Extract:tripolyphosphate ratios of 4:0:2 (Nano F0), 4:1:2 (Nano F1), 4:2:2 (Nano F2), and 4:4:2 (Nano F3).

2.3 Evaluation of Antibacterial Activity

The nanoparticles along with aquadest, zinc bacitracin (10 mg/mL), and free Sargassum sp. Extract (100 mg/mL) were tested for the antibacterial activity using agar well diffusion method [24]. Two bacteria strains, *Escerichia coli* and *Salmonella* sp. Were chosen for evaluation of the antibacterial activity. The bacteria were cultured in nutrient agar medium for 24 h at 37 °C. Bacteria were then harvested and mixed with 5 mL sterile aquadest. After that, 100 μL of bacterial suspensions were mixed with 10 mL semisolid nutrient agar and placed on the Petri dishes. A 5 mm diameter hole was made on the medium with a cork borer. Later on, 100 μL of samples were injected into each well. The Petri dishes were incubated for 24 h at 37 °C. Finally, the inhibition zones were determined by a digital Vernier Caliper.

2.4 Data Analysis

Data analysis were performed by IBM SPSS Statistics 22. Data were analyzed using analysis of variance. Duncan's test was employed to separate means in the variable with significant effect. $P < 0.05$ was considered as significant.

3 Results and Discussion

Antibacterial activity of aquadest, zinc bacitracin, *Sargassum* sp. Extract and four nanoparticles formula against *E. coli* and *Salmonella* sp. Are presented in Table 1. Results showed that the incorporation of *Sargassum* sp. Extract into chitosan-tripolyphosphate nanoparticles provided significant effect on antibacterial activity ($P < 0.05$). F2 has higher *E. coli* and *Salmonella* sp. Inhibition than that of F0 and F1 ($P < 0.05$) but did not differ with F3 ($P > 0.05$). F2 has equivalent *E. coli* inhibition as compared to zinc bacitracin and free *Sargassum* sp. Extract. Moreover, F2 has higher antibacterial activity against *Salmonella* sp. ($P < 0.05$) than that of zinc bacitracin and free *Sargassum* sp. Extract.

Escherichia coli and *Salmonella* sp. Are two common undesirable bacteria in poultry industry. Previous reports have shown that high level of these bacteria in gastrointestinal tract related to the low productive performance, health status, as well as safety of poultry products [25, 26]. Moreover, they were also responsible for the huge economic loss in the poultry industry [27].

Embargo of antibiotics in the global poultry feed industries has forced the use of natural alternatives [28–31]. The current study showed that *Sargassum* sp. Extract could be used as a promising natural antibacterial agent against *E. coli* and *Salmonella sp.* This finding was corroborated by the study of Nofal et al. [32] which also found that

Table 1. Antibacterial activity of *Sargassum* sp. Extract encapsulated into chitosan-tripolyphosphate nanoparticles against *Escherichia coli* and *Salmonella* sp.

Treatments	Inhibition zone (mm)	
E. coli	*Salmonella* sp.	
Aquadest	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Zinc bacitracin (10 mg/mL)	5.80 ± 0.10^{d}	2.73 ± 0.51^{c}
Sargassum sp. Extract (100 mg/mL)	5.47 ± 0.35^{d}	4.53 ± 0.12^{d}
Nano F0*	2.57 ± 0.35^{b}	1.63 ± 0.50^{b}
Nano F1*	3.67 ± 0.12^{c}	2.70 ± 0.20^{c}
Nano F2*	5.60 ± 0.26^{d}	8.40 ± 0.36^{e}
Nano F3*	5.90 ± 0.44^{d}	8.67 ± 0.51^{e}

[ab] uncommon superscripts indicated significant different ($P < 0.05$)

*Nanoparticles were synthesized with the chitosan: *Sargassum* sp. Extract:tripolyphosphate ratios of 4:0:2 (Nano F0), 4:1:2 (Nano F1), 4:2:2 (Nano F2), and 4:4:2 (Nano F3).

Sargassum muticum extract had antibacterial activity against several pathogenic bacteria, including *E. coli* and *Salmonella sp.* Similarly, Park et al. [33] also reported that Sargassum *thunbergii* extracts had antibacterial activity against *E. coli*. In other study, Firdaus et al. [34] observed that the extracts of *Sargassum* spp. Collected from Madura (Indonesia) inhibited the growth of *E. coli* and *Salmonella* sp, with the inhibition zone of 6.10 and 4.10 mm. The antibacterial properties of *Sargassum* sp. Extract is probably linked with the bioactive phenolic compounds. The phenolic compounds had ability to modify membrane potential thus inducing the leakage of cellular constituents [35, 36]. They also could interfere the metabolic routes and DNA synthesis, which in turn will be followed by the cell death [37].

Results in current study showed that nanoencapsulation of *Sargassum* sp. Extracts enhanced the antibacterial activity against *Salmonella* sp. In line with this finding, 'Afifah et al. [38] also reported that *Syzygium polyanthum* leaves extract nanoparticle had greater inhibition zone against *Salmonella typhimurium* when compared to that of the free extract. Ding et al. [39] also found that nanoencapsulation improved antibacterial activity of proanthocyanidin against *Salmonella* sp. Similarly, Maesaroh et al. [40] (2019) also observed that the use of chitosan-tripolyphosphate nanoparticles enhanced antibacterial acitivity of *Annona muricata* Linn leaf extract against *S. typhimurium.* The fundamental reason behind this finding probably attributed to the ability of the nanoparticles to penetrate into bacterial cell walls, resulted the bacterial ultrastructure disruption [39]. Moreover, the use of chitosan-tripolyphosphate nanoparticles could also provided ionic interaction to the anionic surface of bacteria, thereby causing more intensive damage to the cell membrane of pathogens [41]. Together, these mechanisms might provided a greater antibacterial activity to the nanoencapsulated *Sargassum* sp. Extracts.

4 Conclusions

It could be concluded that *Sargassum* sp. Extract encapsulated into chitosan-tripolyphosphate nanoparticles is a promising candidate as an antibiotic alternative in poultry nutrition. Nano-F2 provided the optimum antibacterial activity against *E. coli* and *Salmonella* sp. Among the tested nanoparticles formula.

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