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Antibacterial Activity of Eco Enzyme and Eco Enzyme with *Acorus calamus* Stem Against Contaminated Duck Eggs

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ABSTRACT

In Indonesia, raw duck eggs are often consumed as an additive when consuming herbal medicine. However, duck eggs sold in traditional markets are usually found in dirty conditions, and there is a concern about bacterial contamination. Bacteria in duck eggs could cause diarrhea and typhoid fever. This study aims to identify the presence of *Escherichia coli* and *Salmonella sp.* bacteria in raw duck eggs and perform antibacterial tests using eco enzymes and eco enzymes with *Acorus calamus* stems. In contrast, the test was carried out using the disc diffusion method. The study was conducted on duck eggs from five traditional markets in the city of Medan, and from each market, four duck eggs were taken from three sellers, so the number of eggs studied was 60. Eggshells mashed in a mortar or egg yolks were identified for the number of pathogenic bacteria *Escherichia coli* and *Salmonella sp.* In this study, bacteria were found on eggshells, and an antibacterial test using eco enzyme and eco enzyme with *Acorus calamus stem* was conducted. The parameters measured were zone of inhibition (ZI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The results showed that *Escherichia coli* and *Salmonella sp.* were found in seven of the 60 eggs (11.7%). The bacterial population of *Escherichia coli* were 23 x 10^3 CFU/ml, and Salmonella sp. was 38 x 10^3 CFU/ml, while the population were above the maximum population of the Indonesian National Standard. Eco enzyme with *Acorus calamus stem* has better antibacterial properties than eco enzyme. It has strong ZI, which means it has antibacterial effectiveness on *Escherichia coli* and *Salmonella sp.* MIC start from 3.3%. MBC at a concentration of 10%. In conclusion, eco enzymes with *Acorus calamus* can be antibacterial against *Escherichia coli* and *Salmonella sp* in duck egg shells.

Keywords: Antimicrobial, Contamination, Duck eggs, Eco Enzyme, Inhibition Zone

INTRODUCTION

Food contamination that most often occurs is biological contamination due to exposure to the food, among others, by pathogenic bacteria. Food contaminated with bacteria usually causes health problems (Hennekinne and Dragacci, 2012). One of the foods that are often contaminated with bacteria is duck eggs. The habit of consuming raw duck eggs is widespread in many countries, such as Indonesia, where people usually consume raw duck eggs as an addition to herbal medicine (Laplante, 2016). In England, raw duck eggs are added to salad dressings (Owen et al. 2016). Health problems often reported from consuming raw duck eggs are diarrhea and typhoid fever. In Indonesia, cases of typhoid fever annually average 500 per 100,000 population (Kementrian Kesehatan Republik Indonesia, 2016). In the UK, several outbreaks of Salmonellosis have occurred due to the consumption of duck eggs (Noble et al., 2012).

Duck eggs are often contaminated with pathogenic bacteria, such as those found in Indonesia. For example, in traditional markets in Medan, it isn't easy to find duck eggs in clean conditions. Duck eggs are sold in a dirty state because of soil or duck feces. Research conducted by Nainggolan (2023) on eggs traded in traditional Medan city markets found eggs contaminated with *Escherichia coli*, *Salmonella sp*. and *Shigella sp*. Therefore, bacterial contamination in both shell and yolk is possible. According to Spitzer (2015), several types of *Salmonella* were found to contaminate the shell or penetrate the contents. In Indonesia, *Salmonella typhi* is the most frequently found contaminating duck eggs, which cause typhoid fever to the point of death, although cases of contamination by *Escherichia coli* are also common (Setianingsih et al., 2016; Ayuningtyas et al., 2022).

The contamination of duck eggs is more common in farms, although there are also reports that contamination occurs in an unsanitary

environment when selling eggs. Unhygienic farm conditions or ducks that carry *Salmonella* bacteria are more often the cause of contamination. In the UK and other European countries, ducks are vaccinated to suppress the development of *Salmonella* (Owen et al. 2016). In Indonesia, duck vaccination has never been carried out.

One effort is to clean eggs using antibacterial agents. A food-grade antibacterial agent is a must for human consumers. In this study, the antibacterial that will be studied are eco-enzymes and eco-enzymes with Jeringau (*Acorus calamus*). Eco-enzyme results from fermenting fruits and their skins for 100 days to produce protease, amylase and lipase enzymes (Ginting, 2020; Ginting et al., 2022). These enzymes are antimicrobial because they can kill gram-positive and gram-negative bacteria (Rahman et al., 2020). Fleming and Rumbaugh (2017) stated that protease enzymes can hydrolyze the cell walls of microorganisms. In this study, apart from the eco enzyme itself, the eco enzyme was also used, which was re-fermented using the Jeringau plant (*Acorus calamus*). It is used because it contains secondary metabolites capable of killing bacteria (Balakumbahan et al. 2010; Elshikh et al. 2022). In addition, *Acorus calamus* has fragrance (Hasnah and Fardisa, 2012). This study aims to find an antibacterial that can later be used in cages to clean duck eggs or by homemakers to clean duck eggs before use or storage. Hopefully, the antibacterial can suppress the growth of pathogenic bacteria, both *Escherichia coli* and *Salmonella sp*.

This study has the novelty of other previous studies. In this study, the antibacterial used comes from fermented fruit peels. Jeringau (*Acorus calamus*) is added while Jeringau (*Acorus calamus*) is a simplisia which easily found in traditional markets. The fermentation results contain organic solid acids and enzymes. The use of this fermented product is not only safe for consumers but also solves environmental problems, as fruit peels are waste. Anyone can do the fermentation process, so the antibacterial does not need to be purchased.

MATERIALS AND METHODS

The materials used were nutrient agar (NA) Merck-Germany, Mueller Hinton Agar (MHA) Merck-Germany, Agar Nutri Select Plus (EMB) Sigma-USA, Eosyn Methylene Blue (EMB) Merck-Germany, Salmonella Shigella Agar (SSA) Merck-Germany, aquadest, 70% alcohol, Paper disc, duck eggs.

A selective medium was chosen to detect the presence of *Escherichia coli* and *Salmonella sp*. Another material is the eco-enzyme solution. Apart from that, another material is *Acorus calamus* stems. The material for this research is also the bacteria *Escherichia coli* and *Salmonella sp.,* which were isolated from dirt found on duck egg shells. The tools used are an incubator, OSE, 500 ml spirit lamp, erlenmeyer, autoclave, stirring rod, micropipette, calliper, paper disc, petri dish, and spectrophotometry.

Research Methods

This study proceeded by first collecting duck eggs from five traditional markets in the city of Medan, and from each market, four duck eggs were taken from three sellers so that the number of eggs studied was 60. Egg selection was done randomly as the eggs arrived at the laboratory. It is necessary to carry out the study immediately to reduce the possibility of microbial growth if the eggs are contaminated.

Escherichia coli and *Salmonella sp* bacteria were identified in the eggshell mashed in a mortar. In the shells, if pathogenic bacteria were found, an antimicrobial test using eco-enzyme and eco- enzyme plus Jeringau was performed. Antimicrobial tests were carried out using the disc diffusion method. The parameter measured was the diameter of the zone of inhibition (ZI). Bacterial counts were done manually because the number of bacteria was not too large and visible on the Petri dish. No design was used in this study. The study was conducted descriptively, showing the process of examining contaminated eggs.

Production of Eco enzyme and Eco enzyme plus Jeringau (*Acorus calamus*)

Fruit waste consisting of pineapple and papaya of 1.5 kg each with a total of three kilograms was collected from the traditional market and cut into three cm. 10 litres of untaped water were mixed with one kg of molasses. These fruit wastes were mixed in an airtight container and fermented for 100 days. The extract was filtered and known as an eco enzyme. Three litres of the eco enzyme were mixed with one kg of chopped 3 cm fresh *Acorus calamus* in an airtight container, fermented for one month, then filtered and known as eco-enzyme plus *Acorus calamus*. Eco-enzymes contain enzymes, such as the enzymes in the fruits used to make them.

This research used pineapple and papaya, which contain protease enzymes. The initial fermentation process will grow various microbes that will produce enzymes according to the typology of the substrate.

In this study, re-fermentation was carried out using Jeringau on filtered eco-enzymes. In eco enzymes, ethanol is formed, binding the active ingredients from Jeringau.

This study aims to identify the presence of *Escherichia coli* and *Salmonella sp.* bacteria in raw duck eggs, namely in the shell and egg yolk, and perform antibacterial tests by eco-enzyme and eco-enzyme plus Jeringau (*Acorus calamus*). The results of this study are expected to help increase microbiology knowledge regarding the antibacterial activity of eco-enzyme and eco-enzyme plus Jerigau on duck eggs.

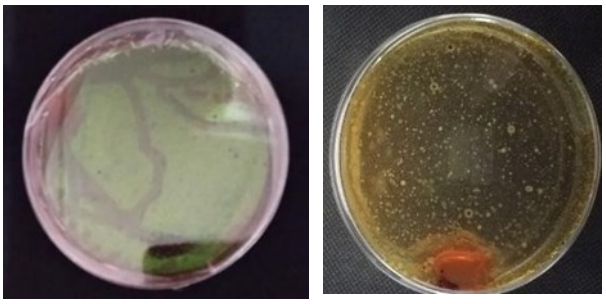
RESULTS AND DISCUSSION

Presence of *Escherichia coli* and *Salmonella sp.* in Duck Eggs

From observations made on 60 samples of duck eggs collected from five traditional markets in Medan, as many as seven duck eggs were positively contaminated by *Escherichia coli* and S*almonella sp*. (11%). Research by Chandran et al. (2016) found that 6.67% of eggs are contaminated with Salmonella. This percentage is at a low level, and according to Chandran et al. (2016), the low level is due to the strict control of hygiene by the farmer and the administration of medication.

Bacteria were found in egg shells, and egg yolks had no bacterial contamination. There were no bacteria in the egg yolk, possibly because the egg sample was two day old. According to Roberts (2004), the longer the dirt sticks to the eggshell, the more likely the bacteria will penetrate the egg, especially if the egg is finely cracked.

Contamination by pathogenic bacteria is generally the result of dirty, undried cage floors. For example, *E. coli* can come from duck feces or be carried by wild birds entering the coop. Therefore, cleanliness and disinfection are necessary. Positive results from *Escherichia coli* contamination were indicated by a color change from purplish red to metallic green. Metallic green colour changes in EMBA because *Escherichia coli* can ferment lactose, increasing the media's acid levels. High acid levels can precipitate methylene blue in EMBA media Lindquist (2004). Yellow and red indicate positive results from Salmonella sp., and H2S is present. The picture of a positive outcome of *Escherichia coli* and *Salmonella sp*. is shown in Figure 1.



A B

Figure 1. The results of the morphological picture of *Escherichia coli* bacterial colonies on Eosin Methylene Blue Agar (EMBA) media (A) and *Salmonella sp* bacterial colonies on Salmonella Shigella Agar (SSA) media (B).

Based on the analysis of the number of *Escherichia coli* and *Salmonella sp* in eggshell and egg yolk samples grown in Eosin Methylene Blue Agar (EMBA) media, data were obtained as shown in Table 1.

Escherichia coli

This study found that the population of *Escherichia coli* was less in egg shells than *Salmonella sp*. However, this number may increase over time. A humid atmosphere is a favourable condition for bacterial growth. Although less, the bacterial population is above the maximum population of the Indonesian National Standard. By regulations or supervision for the protection of consumers regarding animal quality products circulating through the Indonesian National Standard SNI No. 01-6366-2000 concerning the maximum limit of microbial contamination in fresh eggs is $1x10^1$ colonies per g (BSN, 2000) while in this study, the population of *Escherichia coli* was $23x10^3$ CFU/ml on 10^{-3} dilution. In this study, colony population calculations were done manually. A dilution of 10-3 causes the colony population looksrare, so it is easy to do manual calculations. *Escherichia coli* bacteria live in the digestive tract of humans and animals; therefore, these bacteria in eggs indicate the presence of these bacteria in eggs, which indicates unhygienic conditions in the duck-rearing environment. Humans infected with *Escherichia coli* will have health problems because *Escherichia coli* can grow excessively

and become pathogenic if contained in large quantities. Consumption of raw eggs, such as in herbal medicine sellers where a mixture of herbal medicine and duck egg has the temperature and pH ideal for the growth of *Escherichia coli*, causes the slightest contamination of *Escherichia coli* to support the development of these bacteria. According to Ingraham and Ingraham (2004), the optimal growth of *Escherichia coli* is at a temperature of 35°C-37°C and a pH of 7-7.5, which supports the bacteria to grow excessively.

Salmonella sp

In this study, a larger population of *Salmonella sp* was found in the eggshell. *Salmonella sp.* causes salmonellosis, a disease with diarrhea, nausea, vomiting, and fever symptoms that can lead to death. Because the effect of *Salmonella sp.* is more severe than *Escherichia coli,* the Government has issued a regulation of the Indonesian National Standard SNI No. 01-6366-2000 that the Salmonella population should be zero. In this study, the *Salmonella sp.* population was 38 x 10^3CFU/ml.

According to Shivaning Karabasanavar et al. (2020), Salmonella serotypes commonly found in poultry are *Salmonella typhimurium, Salmonella enteridis, Salmonella infantis and Salmonella pullorum*. *Salmonella typhimurium* is the most common cause of food-borne diseases in humans. Calculation of the colony population in this study was done manually. This is due to the 10-3 diluent; the appearance of colonies in petri dishes is rare, so it is easy to count them. Colony numbers are shown in Table 1.

Even though Salmonella colonies were found on the egg shells, they were not found in egg yolks. This is probably because the research eggs are still new. The egg's contents are protected from bacteria by two membranes under the outer shell layer, but gram-negative bacteria can still penetrate the shell and egg membrane and grow inside the egg (Madigan et al., 2014; Messens et al. 2006). As the egg ages, bacteria can enter the egg through the enlarged pores. This is what causes stored eggs to be more susceptible to contamination. The optimal temperature for *Salmonella sp*. growing is 6.7°C–45°C, and it stops growing at 5°C. At a temperature of 55°C, it can survive for one hour; at a temperature of 60°C, it can live for 15-20 minutes (Ray, 2004).

Table 1. The number of *Escherichia coli* and *Salmonella sp.* colonies in duck eggshell (CFU/ml).

No.	Samples	The number of colonies of *Escherichia coli* (CFU/ml)				
		Dilution 10^{-1}	Dilution 10^{-2}	Dilution 10^{-3}	Dilution 10^{-4}	Dilution 10^{-5}
(1)	Duck eggshell	Un countable	Un countable	23 x 10^3	15 x 10^4	10 x 10^5
	Duck egg yolk	0	0	0	0	0
(2)	Duck eggshell	Un countable	Un countable	25 x 10^3	20 x 10^4	8 x 10^5
	Duck egg yolk	0	0	0	0	0
(3)	Duck eggshell	Un countable	Un countable	21 x 10^3	10 x 10^4	12 x 10^5
	Duck egg yolk	0	0	0	0	0
(4)	Duck eggshell	0	0	0	0	0
	Duck egg yolk	0	0	0	0	0
(5)	Duck eggshell	Un countable	Un countable	22 x 10^3	14 x 10^4	11 x 10^5
	Duck egg yolk	0	0	0	0	0
(6)	Duck eggshell	Un countable	Un countable	24 x 10^3	16 x 10^4	9 x 10^5
	Duck egg yolk	0	0	0	0	0
(7)	Duck eggshell	Un countable	Un countable	23 x 10^3	15 x 10^4	10 x 10^5
	Duck egg yolk	0	0	0	0	0

Antibacterial Test with Eco enzyme and Eco enzyme Plus Jeringau (*Acorus calamus*)

Antibacterial tests have been carried out on *Escherichia coli* and *Salmonella sp*. bacteria using eco-enzyme and eco-enzyme plus Jeringau (*Acorus calamus*). Previously, eco enzyme and eco enzyme plus Jeringau were diluted with ratios of 1: 10 (concentration 10%), 1: 20 (concentration 5%) and 1:30 (concentration 3.3%). The power of antibacterial is reflected in the clear zone area. The results of the antibacterial test are shown in Table 2.

The Minimum Inhibitory Concentration (MIC) of Eco enzyme and Eco enzyme plus Jeringau (*Acorus calamus*) on *Escherichia coli* and *Salmonella sp* is a concentration starting at 3.3% (dilution 1:30). At a concentration of 3.3%, the inhibition zone formed is about 7.1. However, this inhibition zone was still smaller than the control positive using Kanamycin, with an inhibition zone of 10 mm.

The MBC test (Minimum Bactericidal Concentration) aims to determine the minimum concentration of antigens that cause the absence of colony growth in a petri dish. In this research, the MBC Test, which is presented in Table 3, show that at a concentration 3.3% of Eco enzyme and Eco enzyme plus Jeringau (*Acorus calamus*), there are no colonies of *Escherichia coli* and *Salmonella sp* at 10^4 colonies population while then the number is zero at 10^3 colonies population with 100% concentration.

Research conducted by Rahmawati and Bintari (2014) found a clear zone of 6.86 mm from applying 100% Binahong Leaf (*Anredera cordifolia*) against *Salmonella*. Abima (2017) found a clear zone by using 25%, 50%, and 75% of Binahong Leaf extract 11.86 mm, 13.75 mm and 15.41mm, respectively, against *Escherichia coli*. Rita et al. (2017), using the essential oil of Jeringau (*Acorus calamus*), found an inhibition of 11.33 mm on the growth of *Escherichia coli*. Rahman et al. (2020) conducted an eco-enzyme antagonist test against *E.coli*. They found a clear zone of 8 mm. Geeta and Kaparapu (2017) found that the antimicrobial activity of eco-enzymes from orange peel extract showed an excellent inhibition zone against *E. Coli* of 11 mm. Orange peel contains the enzyme pectinase.

The clear zone indicates the ability to inhibit bacterial growth. Eco enzymes have a clear zone, especially 100% extract, and this ability is due to the content of enzymes, including protease enzymes that can hydrolyse bacterial cell walls. In this study, eco enzymes were made by fermenting papaya and pineapple, which are rich in protease enzymes.

Eco enzyme enriched with Jeringau produces an even stronger clear zone. This is because, in Jeringau, there are antibacterial secondary metabolites such as alkaloids, flavonoids and polyphenols. Rahman et al. (2020) found that the application eco enzyme has a smaller clear zone. This is probably because Rahman et al. (2020) made eco enzymes from potato skins, pumpkin and cabbage, so amylase is mostly available.

Table 2. Inhibition zone measurement of Eco enzyme and Eco Enzyme Plus Jeringau (*Acorus calamus*) results against *Escherichia coli* and *Salmonella sp.*

No	Treatments	Antimicrobial on	Zone of inhibition (mm)
1	Eco enzyme 100%	*Escherichia coli*	9.3
2	Eco enzyme 1: 10	*Escherichia coli*	8
3	Eco enzyme 1: 20	*Escherichia coli*	7.7
4	Eco enzyme 1: 30	*Escherichia coli*	7.3
5	Eco enzyme 100%	*Salmonella sp.*	9.8
6	Eco enzyme 1: 10	*Salmonella sp.*	8.9
7	Eco enzyme 1: 20	*Salmonella sp.*	7.7
8	Eco enzyme 1: 30	*Salmonella sp.*	7
9	Eco enzyme + Jerango (*Acorus calamus*) 100%	*Escherichia coli*	9.2
10	Eco enzyme + Jerango (*Acorus calamus*) 1:10	*Escherichia coli*	8.1
11	Eco enzyme + Jerango (*Acorus calamus*) 1:20	*Escherichia coli*	7.8
12	Eco enzyme + Jerango (*Acorus calamus*) 1:30	*Escherichia coli*	7.6
13	Eco enzyme + Jerango (*Acorus calamus*) 100%	*Salmonella sp.*	11.5
14	Eco enzyme + Jerango (*Acorus calamus*) 1:10	*Salmonella sp.*	8
15	Eco enzyme + Jerango (*Acorus calamus*) 1:20	*Salmonella sp.*	7.5
16	Eco enzyme + Jerango (*Acorus calamus*) 1:30	*Salmonella sp.*	7.1

Binahong leaf application also produces a clear zone due to the content of secondary metabolites such as flavonoid and antibacterial saponin compounds. Fleming and Rumbaugh (2017) mentioned that the protease enzyme can hydrolyze the bacterial cell wall.

According to Vasantha Kumari et al. (2009), the inhibitory zone on microbes reflected inhibitory power. There were three categories of

inhibitory or antimicrobial zone, i.e. very strong if the inhibitory zone >12 mm, strong 8-12 mm and weak < 8 mm. In this research, the inhibitory zone of the application of eco enzyme and eco enzyme plus Jeringau is classified as strong for 100% and 10% of *Escherichia coli* and *Salmonella sp*. However, there is a tendency for eco enzyme plus Jeringau to have more potent antimicrobial properties than eco enzyme alone.

In eco enzymes made from fruits, there are several enzymes such as lactase, amylase, and protease. Fruits that carry protease enzymes, such as papaya and pineapple, cause a higher concentration of protease enzymes. The protease enzyme can hydrolyse the bacterial cell wall. That is why the antibacterial test in this study at a concentration of 100% produced a stronger antimicrobial zone than the research by Rahman et al. (2020), which made eco enzymes from potatoes, cabbage and pumpkin.

Eco enzyme plus Jeringau (*Acorus calamus*) produces an even stronger antimicrobial zone. This is because there are secondary metabolites from Jeringau, which are potent antimicrobials, in eco enzyme plus Jeringau. This anti-bacterial study was also conducted using chloramphenicol antibiotics, and an inhibition zone of 28.1 mm was obtained. A very strong inhibition zone of Chloramphenicol because it is a broad-spectrum commercial antibiotic.

According to Kumar (2013), *Acorus calamus* has better antimicrobial abilities for methanol extracts compared to other solventextracts such as *B. edulis*, *C. Bondicella, C.orchioides, H. isora, H. pubescens, P.sativa, P. integerrima, Q. infectoria, R. serpentina, S. lappa, T. stocksianum, and X. strumarium*. All these are medicinal herbs that are antimicrobial and widely used as medicine in India. *Acorus calamus* contains active ingredients of flavonoids, alkaloids, phenolic compounds, tannins, steroids, saponins, glycosides and terpenoids. Miao et al. (2016) stated that *Acorus calamus* has antibacterial properties that can be used for food preservation. Because the *Acorus calamus* is antimicrobial, it is hoped that the eco enzyme plus the *Acorus calamus* will have even better antimicrobial abilities than the eco enzyme. Livestock is prone to bacterial contamination. Bacterial contamination often occurs in ducks kept in cages with dirt floors, like those found in Indonesia. This is because the cage with a dirt floor is difficult to clean and further disinfect. Dirt attached to the eggshell can carry bacteria. Dirt must be cleaned before the product is marketed, for example, by cleaning eggs using an antibacterial solution.

From the explanation above, it is known that eco enzyme and eco enzyme plus Jeringau can inhibit the growth of bacteria. Eco and eco enzymes, plus Jeringau, have added value because they are made from fermenting fruit waste, making this product environmentally friendly. Making eco enzymes does not require significant costs, and the technology is simple (Ginting, 2020) so that duck farmers can make it themselves.

Table 3. Number of *Escherichia coli* and *Salmonella sp*. colonies treated with eco enzyme and eco enzyme plus Jeringau (*Acorus calamus*)

No.	Treatments	Dilution 10^{-3}	Dilution 10^{-4}	Dilution 10^{-5}
	Escherechia coli colonies (CFU/ml)			
1.	Ecoenzyme 10%	10	3	0
2.	Ecoenzyme 5%	14	0	0
3.	Ecoenzyme 3.3%	19	5	0
4.	Ecoenzyme 100%	0	0	0
	*Salmonella sp.*colonies (CFU/ml)			
5.	Ecoenzyme + Jeringau (*A. calamus*) 10%	8	1	0
6.	Ecoenzyme + Jeringau (*A. calamus*) 5%	18	0	0
7.	Ecoenzyme + Jeringau (*A. calamus*) 3.3%	15	4	0
8.	Ecoenzyme + Jeringau (*A. calamus*) 100%	0	0	0

In addition, Jeringau adds a distinctive fragrance. Eco enzyme plus Jeringau can be a good alternative as an antibacterial agent because it can strongly inhibit the growth of bacteria.

CONCLUSION

Eco enzyme plus Jeringau (*Acorus calamus*) has a more vital antibacterial zone than the only eco enzyme. ZI by 100 % eco enzyme was 9.3 mm on *Escherichia coli* and 9.2 mm on *Salmonella sp.* while eco enzyme plus *Acorus calamus* was 9.2 mm on *Escherechia coli* and 11.5 mm on *Salmonella sp*. Eco enzyme plus Jeringau (*Acorus calamus*) has antibacterial effectiveness on *Escherichia coli* and *Salmonella sp.* MIC start from 3.3%. MBC at a concentration of 10%. It could be used as an antibacterial on duck eggs.

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REFERENCES

Ayuningtyas, G., Martini, R., & Yulianti, W. (2022). The Role of Dipping Duck Hatching Eggs with Cherry Leaf Extract as Natural Sanitizers on Hatching Performance and Eggshell Bacterial Counts. In E3S Web of Conferences (Vol. 348, p. 00023). EDP Sciences.

Badan Standardisasi Nasional SNI 01-6366-2000. Batas Maksimum Cemaran Mikroba dan batas maksimum residu dalam bahan makanan asal hewan.

Balakumbahan R, Rajamani K, Kumanan K. 2010. Acorus calamus: An Overview. Journal of Medicinal Plants Research. 4(25): 2740-2745.

Chandran, T T., Aini Ideris and Saleha Abdul Aziz. Salmonella, Escherichia coli and Coliform Contamination of Layer Chicken Eggs From Farm To Market. Universiti Putra Malaysia Press Serdang• 2016, 162.

Elshikh, M.S., E. Rani, D.A. Al Farraj, F.M.A. Al-Hemaid, M.R.Abdel Gawwad, T.R.J. Jeba Malar, L. Dyona, P. Vijayaraghavan. 2022. Plant secondary metabolites extracted from Acorus calamus rhizome from Western Ghats, India and repellent activity on Sitophilus oryzae. Physiological and molecular plant pathology. Volume 117: pp 101743.

Abima, F. M. Bahar, A. Chairani. 2017. Uji Efektivitas Antibakteri Ekstrak Daun Binahong (Anredera cordifolia (Ten.) Steenis) terhadap Isolat Bakteri Escherichia coli Jajanan Cilok Secara In Vitro dengan Metode Diffusi. J.Profesi Medika. 11 (10): 1-6.

Fleming, D. & K.P. Rumbaugh. 2017. Approaches to dispersing medical biofilms. Micro-organism 5(2):1-16. DOI: 10.3390/microorganisms5020015.

Geetha, S. and J. Kaparapu. 2017. Antimicrobial activity of fermented citrus fruit peel extract. Int Journal of Engineering Research and Application. 7: 25-28.

Ginting, N. 2020. Financial analysis of GE (garbage enzyme) application at Universitas Sumatera Utara campus. Sustainable Campus Effort during the Covid-19 Pandemic. Managing Sustainable Universities During Covid-19 Pandemic. Undip Press. Semarang.

Ginting, N., Hasnudi, Yunilas, and L. Prayitno. 2022. Dilution of eco enzyme and antimicrobial activity against Staphylococcus aureus. JITRO (Jurnal Ilmu dan Teknologi Peternakan Tropis). 9(1):123-128.

Hasnah, Husni and Ade Fardhisa. 2012. Pengaruh Ekstrak Rimpang Jeringau (Acorus Calamus l.) Terhadap Mortalitas Ulat Grayak Spodoptera Litura F. J.Floratek 7 115-124.

Hennekinne, J., M.De Buyser and S. Dragacci. 2012. Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiology Rev. 36(4): 815-836.

Ingraham, J. L., & Ingraham, C. A. (2004). Introduction to microbiology: a case history approach. (No Title).

Kementerian Kesehatan Republik Indonesia. 2016. Situasi Diare di Indonesia. Jakarta: Buletin Jendela Data dan Informasi Kesehatan.

Kumar, A., Vandana. 2013. Medicinal Properties of Acorus calamus. Journal of Drug Delivery &Therapeutics. 3 (3): 143-144.

Laplante, J. (2016). Becoming-plant: jamu in Java, Indonesia. Plants and health: new perspectives on the health-environment-plant nexus, 17-65.

Lindquist, J. 2004. Deferensial Media Eosin Metheyline Blue Agar 4(2):61-65 http://www.jildiuist.net/generalmiscro/defb.html.

Madigan, M.T., J.M.Martinko, K. S. Bender, D.H. Buckley, D.A. Stahl. 2014. Brock Biology of Microorganisms, 14th ed. Glenview: Pearson Education.

Messens, W., K. Grijspeerdt, L. Herman. 2006. Egg shell penetration of hen's egg by Salmonella enterica serovar Enteridis upon various storage conditions. British Poultry Science . 47(5) : 554-560.

Miao, J., R. Shi, C. Li, X. Li, Q. Chen. 2016.Essential Oils in Food Preservation, Flavorand Safety. Chapter 88- Sweet Flag (Acorus calamus) Oils. Academic Press. 2016, Pages 775-782.

Nainggolan, N. N. (2023). Isolasi Bakteri Pengkontaminasi Telur Ayam yang Diperjualbelikan di Pasar Tradisional di Kota Medan dan Sekitarnya. https://repositori.uma.ac.id

Noble, D.J., C.Lane, C.L. Little, R. Bavies, E.De Pinna, L. Larkin and D. Morgan. 2012. Revival of an old problem: an increase in Salmonella enterica serovar. Epidemiol Infact.140 (1): 146-149.

Nor Faiza, S., Saleha, A. A., Jalila, A., & Fauziah, N. (2013). Occurrence of Campylobacter and Salmonella in ducks and duck eggs in Selangor, Malaysia. Tropical Biomedicine 30(1): 155–158

Owen, M., F. Jorgensen, C. Willis, J. McLauchlin, N. Elviss, H. Aird, A. Fox,M. Kaye, C. Lane and E. de Pinna. 2016. The occurrence of Salmonella spp. in duck eggs on sale at retail or from catering in England. Letters in Applied Microbiology 63: 335-339.

Rahman, S., I. Haque, R.C. Dev Goswami, P. Barooah, K. Sood, and B. Choudhury. 2020. Characterization and FPLC Analysis of Garbage Enzyme: Biocatalytic and Antimicrobial Activity. Waste and biomass valorization 12(1): 1293-302.

Ray, B. 2004. Fundamental Food Microbiology.New York: CRC Press.

Rahmawati, F. and S. H. Bintari. 2014 Studi Aktivitas Antibakteri Sari Daun Binahong (*Anredera cordifolia*) Terhadap Pertumbuhan Bacillus cereus dan Salmonella enteridis. Unnes Journal of Life Science. 3 (2): 103-111.

Rita, W. S., I. W. Suirta, and P. Utami. 2017. Aktivitas Antibakteri Minyak Atsiri Rimpang Jeringau (Acorus calamus) terhadap Bakteri Escherichia coli dan Staphylococcus aureus. Cakra Kimia. 5 (2) : 130-136.

Roberts, J.R. 2004. Factors Affecting Egg Internal Quality and Eff Shell Quality in Laying Hens. The Journal of Poultry Science. 41(3): 161-177.

Setianingsih, I., Y., Saputra, D. C. Arianti. 2016. Kontaminasi Enterobacteriaceae pada telur itik Alabio di Kabupaten Hulu Sungai Utara, Kalimantan Selatan. Journal of Health Epidemiology and Communicable Diseases. 2 (2): 52-58.

Shivaning Karabasanavar, N., Benakabhat Madhavaprasad, C., Agalagandi Gopalakrishna, S., Hiremath, J., Shivanagowda Patil, G., & B Barbuddhe, S. (2020). Prevalence of Salmonella serotypes S. Enteritidis and S. Typhimurium in poultry and poultry products. Journal of Food Safety,40(6), e12852.

Spitzer, H. 2015. Celebrating Scholarship and Creativity Day. An Analysis of Bacterial Contamination of Chicken Eggs and Antimicrobial Resistance. P. 3-37.

VasanthaKumari, N., A.S. D.Alshrari, E.G.Rad, H.G. Moghaddam, A. van Belkum, M.A. Alreshidi, N. Selamat, M.N. Shamsudin. 2009. Highly dynamic transient colonization by Staphylicoccuc aureus in healthy Malaysian student. J Med Microbiol. 58 (11): 1531-1532.