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The Benefit of Society for Antibiotic Growth Factors Using Probiotics and In Vitro Leaf Extracts

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Abstract

The increasing demand for chicken meat resulted in increased chicken production. To determine the bland power of probiotic antibacterial activity (B. subtilis and L. acidophilus) against E. coli growth through Minimum Inhibitory Concentration (MIC) and Minimum Bacterisidal Concentration (MBC), to determine the bland force of antibacterial activity of ethanol extract E. scaber linn against E. coli growth through Minimum Inhibitory Concentration (MIC) and Minimum Bacterisidal Concentration (MBC) and to identify the activity of probiotics and ethanol extract E. scabber Linn as an alternative to antibiotic growth promoters. This study is an experimental descriptive study. The dried samples of tapak liman (Elephantophus scaber L.) leaves were weighed as much as 700 grams and put into a maceration container. Simplicia was macerated using 2 types of solvents, namely 75% ethanol and 96% ethanol, so that 2 types of ethanol extract were obtained. Based on the results of disk diffusion and optical density tests analyzed with statistical testing. For data from the results of the disk diffusion method analyzed with One-Way Analysis of Variance (ANOVA) and continued with Post-Hock, data from the analysis with dilution methods were analyzed with Kruskal-Wallis and continued with Mann-Whitney, while MBC dinalysis results were descriptive. Research results: Apart from green tea and reeds, other plants such as tapak liman also have antibacterial activity. The combination of tread liman with chloramphenicol against E. coli has additive value. Previous tests used extracts from tapak liman which were still in the form of crude extracts, namely alkaloids, saponins, terpenoids, flavonoids, steroids, tannins and quinones that would interact with antibiotics that have antibacterial activity (Dwary et al., 2020), because E.scaber contains compounds flavonoids, tannins, saponins and alkaloids. According to the results of the study, the results of the MIC showed 10% for Gynura Procumbens leaf extract, and 30% for E. scaber leaves. The results of the LDH study showed that there was an inhibition of 96% ethanol extract of G. procumbens and E. scaber leaves on the growth of S. thypi bacteria with the inhibitory power in the weak category. For G. procumbens leaf extract the highest LDH was 4.5 mm at 30% concentration and the highest LDH for E. scaber leaf extract was 4.3 mm at 50% extract concentration.

Keywords
age; gender; length of work; attitude; adherence to procedures

I. Introduction

Meat is one of the products of livestock origin that can contain chemical hazards, namely antibiotic residues (Mukti, 2017). The presence of antibiotic residues in meat is due to farmers not complying with the dosage (Bahri et al., 2005) and discontinuation of

medication. The presence of antibiotic residues that exceed the maximum residue limit (BMR) makes meat unsafe for consumption (Ngangguk et al., 2014). Safe food quality control can be done by identifying pathogenic microbes in foodstuffs of animal origin, such as *Escherichia coli* (Nisa et al., 2018).

The problem of antibiotic resistance is very complex that occurred in Indonesia and will continue to increase every year. Infectious disease reaches more than 13 million deaths per year in the country develop. (BPOM, 2011) Disease infection in Indonesia is still included in the top ten diseases, thenby 2050 deaths from Antibiotic resistance reaches 10 million per year and is the cause of death highest among other causes. Use of antibiotics in Indonesia which is quite high and lacking right will increase the incidence resistance (Ministry of Health, 2011b).

Bacterial resistance level in Indonesia continues to increase, according to Resistance Control Committee Antimicrobial from 2013, 2016, until 2019. The resistant bacteria are getting up from 40 percent, 60 percent, and 60.4 percent in 2019. Increase The incidence of resistance is caused by Inappropriate use of antibiotics under control. Resistant bacteria can occur due to misuse of antibiotics (Ministry of Health, 2011a).

Antibiotics work cytostatically or cytotoxic to eliminate microorganisms (Fathonah, 2018). The main cause of antibiotic resistance is the unwise use of antibiotics in humans and animals (Negara, 2016). The impact of antibiotic resistance is that treatment efforts become more difficult and require higher health costs (Utami, 2011).

Antibiotics as a growth promoter material (Andriani et al., 2020) used to increase growth (Haryanto, 2013) and broiler chicken production and disease prevention. In this study, an extraction process by maceration will be carried out to obtain flanovoid luteolin which is thought to have antibacterial activity.

Based on the background of the problems that have been stated, researchers are interested in researching probiotics and tapak liman leaf extract as an alternative to AGP (Antibiotic Growth Promoter). Researchers wanted to compare the activity of probiotics and ethanol extracts (75% and 96%) of tapak liman leaves in inhibiting the growth of *E. coli*. This test was carried out with the hope that probiotics or tapak liman leaves could be a solution to suppress the rate of resistance due to uncontrolled and unsupervised use of antibiotics.

This study aims to determine the inhibition of the antibacterial activity of probiotics (*Bacillus subtilis* and *Lactobacillus acidophilus*) on the growth of *E. coli* through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), to determine the inhibition of antibacterial activity of *Elephanthopus scaber* linn ethanol extract on growth of *E. coli* via MIC and (MBC) and to identify the activity of probiotics and ethanolic extracts of *E. scabber* Linn as an alternative to AGP. This study is expected to provide a scientific description and explanation of the antimicrobial effects of *E. scabber* and probiotics as well as their ability as candidates for growth promoters in vitro and it is hoped that this research will add information and supporting data for further research in the context of developing alternative uses for natural ingredients. as a substitute for AGP.

II. Research Method

This research is an experimental research. The aims of the study were to determine the effect of *E. scabber* Linn on the inhibition zone of *E. coli*, and to determine the growth of probiotics (*B subtilis* and *L acidophilus*) on the growth inhibition of *E. coli.*

Sampling was carried out at the Laboratory of the Faculty of Pharmacy, Airlangga University. The research variables consisted of independent variables, namely the concentration of tapak liman extract, *B subtilis*, *L. acidophilus*, control variables namely plant species (*E. scaber* Linn), agar media, plant parts taken (leaves), response variables namely growth of *E. coli* bacteria. The confounding variables are the age of the plant, the environment in which it grows, the time of harvesting, this variable is a variable that must be controlled, because it has an influence on the research even though it is not investigated.

The tools used in this study were petri dishes, test tubes, spatula, konical, autoclave, incubator, refrigerator, tweezers, ose, Bunsen, microscope, object glass, syringe, microplate reader, caliper, centrifuge and vortex. The materials used were *E. coli*, ATCC®, 1175 (pathogenic bacteria) and *B. substilis* ATCC®, 6633 (Probiotic bacteria) obtained from Veterinary Medicine (BVET), and *L. acidophilus* (Probiotic bacteria) obtained from the Food and Nutrition Culture Collection. Broth Hearth Infusion (BHI, Merck TM), Phosphate buffered saline (PH 7.4 Sigma TM), Mueller Hilton agar (MHA, Merck TM), Nutrient Agar (Merck TM), H202, oxidase paper, indole motility sulfide medium (SIM), Merck TM), indole, methyl-red, voges-proskauer citrate (IMVIC), urea media, carbohydrates, (d-glucose, lactose, d-mannitol, d-sorbitol, d-maltose, d-sucrose), aqudest, DMSO 10%, ethanol extract 75% tapak liman leaves, ethanol extract 96% tapak liman leaves, blank disk (Oxoid TM), chloramphenicol antibiotic. This research will be carried out from July to September 2021 in the Pharmaceutical Biotechnology laboratory, Faculty of Airlangga University, and will be carried out in July 2021 - September 2021.

III. Results and Discussion

The ability of lactic acid bacteria probiotic microbes to suppress the growth of pathogenic bacteria (Nurainy & Rizal, 2018) This is due to its ability to produce antimicrobial compounds such as lactic acid and acetic acid (Rusmana et al., 2012), considerable hydrogen peroxide and bacteriocin (Afrimansyah et al., 2014). The accumulation of these compounds in cells occurs because lactic acid bacteria do not produce the enzyme catalase (Hamidah et al., 2019). The antimicrobial effect of lactic acid bacteria is caused by the metabolite of lactic acid bacteria in the form of undissociated lactic acid (Kusumawati, 2012), the dissociation constant (pKa) of lactic acid is 3.8 (Pikoli & Yunita, 2018). Antimicrobials from undissociated molecules are produced by the dissociation of molecules in the cytoplasm which then enter the cell through the membrane (Halim & Zubaidah, 2013). The release of H+ protons after dissociation begins with a decrease in the trans-membrane proton gradient, neutralization of the proton-motive force, a decrease in internal pH which can cause denaturation and loss of cell viability.

The four types of LAB used had antibacterial activity against *B. cereus, E. coli,* and *S. aureus*. Research result (Akpinar, A., 2011) showed the ability of all *L. bulgaricus* strains isolated from home-produced yogurt in Turkey to suppress the growth of *E. coli,* some strains were able to suppress the growth of *B. cereus, Kl. pneumoniae, L. monocytogenes, B. coagulant* and *S. aureus*. In addition to its ability to produce lactic acid and hydrogen peroxide, *L. bulgaricus* also produces bacteriocins known as bulgarican. This compound is able to suppress both gram-positive and gram-negative pathogenic bacteria. Most of the *S. thermophillus* strains in the study were also able to suppress the growth of the pathogenic bacteria *Kl. Pnomanie, B. coagulans, P fluorescens, E. coli, S. aureus* and *L. monocytogenes*. Some strains of *S. thermophillus* were able to suppress the

growth of *B. cereus*. Bacteria *L acidophillus* and *L. casei* were also reported to have antibacterial activity against Methicillin Resistant Staphylococcus Aureus (MRSA). *L. acidophillus* is reported to produce bacteriocin acidophilucin A (Quwehand and Vesterlund in Kaboosi, 2011) and non-lactic acid anti-bacterial compounds (not yet identified) which can inhibit the growth of pathogenic bacteria such as *S. aureus, L monocytogenes, S. Typhimurium, P. aeruginosa* and *Enterobacter cloacae*.

The fractionation carried out aims to separate the crude extract of tapak liman in several metabolites which will then be tested for its inhibitory power with a combination of antibiotics. One of the studies on the antibacterial activity of the ethanolic extract of tapak liman, found that the fractionation has different antimicrobial potential according to the compounds contained in it which can also be different. Tread liman fractionation can have different types of interactions with previous findings (Boesary, 2019). In addition to green tea and reeds, other plants such as tapak liman also have antibacterial activity (Dwary et al., 2020). The result of this research is that the combination of tread liman with chloramphenicol against *E. coli* has additive value. Previous tests used extracts from tapak liman which were still in the form of crude extracts, namely alkaloids, saponins, terpenoids, flavonoids, steroids, tannins and quinones that would interact with antibiotics that have antibacterial activity (Dwary et al., 2020), because *E. scaber* contains flavanoid compounds, tannins, saponins and alkaloids. According to the results of the study, the MIC results showed 10% for *G. procumbens* leaf extract, and 30% for *E. scaber* leaves. The results of the LDH study showed that there was an inhibition of 96% ethanol extract of *G. procumbens* leaves and *E. scaber* leaves on the growth of *S. thypi* bacteria with the inhibitory power in the weak category. For *G. procumbens* leaf extract the highest LDH was 4.5 mm at 30% concentration and the highest LDH for *E. scaber* leaf extract was 4.3 mm at 50% extract concentration.

Various kinds of fermented milk products have been claimed as probiotic products, namely food ingredients containing live microbes that have beneficial effects on the health of the public interest. In general, probiotics are lactic acid bacteria. The most widely used probiotic cultures in dairy products are Lactobacillus and Bifidobacteria. In fermented milk, the dominant microbe is a group of lactic acid bacteria. During the fermentation process, bacteria will produce metabolites that can cause changes in taste and shape in milk and are able to inhibit the growth of destructive and pathogenic bacteria. Lactic acid bacteria, which are probiotics, have the ability to survive and form colonies in the intestines then produce lactic acid and bacteriocins and stimulate the immune system. Lactic acid bacteria play a role in maintaining the balance of intestinal flora and help boost the immune system, which is beneficial for the benefit of the community to maintain health.

IV. Conclusion

The ability of probiotic lactic acid bacteria to suppress the growth of pathogenic bacteria. The antimicrobial effect of lactic acid bacteria is caused by the metabolite of lactic acid bacteria in the form of undissociated lactic acid. In addition to green tea and reeds, other plants such as tapak liman also have antibacterial activity. The result of this research is that the combination of tread liman with chloramphenicol against *E. coli* has additive value. Previous tests used extracts from tapak liman which were still in the form of crude extracts, namely alkaloids, saponins, terpenoids, flavonoids, steroids, tannins and quinones that would interact with antibiotics that have antibacterial activity.

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