Antimicrobial effects of chive extracts against bacteria pathogen and *Lactobacillus acidophilus*

Dewi Restuana Sihombing¹, Herla Rusmarilin², Dwi Suryanto², Sanggam Dera Rosa Tampubolon¹, Sisilia Florina Yanti¹

¹Faculty of Agriculture, Universitas Katolik Santo Thomas, Indonesia
²Faculty of Agriculture, Faculty of Math and Science, Universitas Sumatera Utara, Indonesia

E-mails: *dewirestuanasihombing@gmail.com, herla_surabaya@yahoo.com*

**Abstract.** Chive (*Allium schoenoprasum* L) is one type of nutritious plant that can be used as an antimicrobial. This study aims to examine the antimicrobial activity of extracts chive. Extraction was done by maceration, such as using water solvent, methanol, ethyl acetate, and comparative solvents, namely dimethyl sulfoxide as a negative control and tetracycline as a positive control. The sample bacteria used were *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Lactobacillus acidophilus*. This study used disc diffusion method, with a complete 2-factor random design and 5 replications. The research finding showed that extracts chive with a concentration of 20%, 40%, 60% and 80% had an effect on the growth inhibition of *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, while *Lactobacillus acidophilus* bacteria did not show any inhibition zone, based on the research finding. It was known that chive extract is one of the good media for the growth of *Lactobacillus acidophilus* bacteria, so extracts chive is very potential for the development of food products, especially those related to probiotics.

**Keywords:** chive extract, antimicrobial, pathogenic bacteria, probiotic bacteria

1. Introduction

Chive is one type of plants in North Sumatra, which is often used as an additional ingredient in familiar food of Batak, namely arsik. Chive plants or commonly known by the people of North Sumatra as "batak onions" have been used in every generations as a flavoring dish. The taste is distinctive and the smell is fragrant makes the taste of cuisine more delicious. Chive plant is a one member of the Liliaceae tribe. According to Hegnauer's hypothesis, plants that come from the same tribe have similar chemical patterns. This case is based on the phytoequivalent hypothesis which stated that plants with the same chemical patterns have the same activity, so based on this hypothesis, it was expected that chive plants from the same tribe (*Liliaceae*) have similar activities to another onions [1,2]. One type of onion that is often used as an antimicrobial is garlic. Garlic has a broad antimicrobial spectrum that can kill negative bacteria and positive bacteria. Alisin is an active substance in garlic which is effective in inhibiting microbial growth [3].
Based on previous research on garlic extract, it was known that the secondary metabolite content in garlic has antimicrobial activity against some pathogenic bacteria. Antimicrobial activity of garlic has been examined in four bacteria, namely: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Based on the research, inhibition zones were obtained in each bacterium, namely: 15 mm, 17 mm, 20 mm, and 8 mm [4]. Pathogenic bacteria such as *Escherichia coli*, *Shigella dysenteriae*, and *Staphylococcus aureus* are diarrhea-causing bacteria that often infect humans. One of the triggers of dysentery is infection by bacteria, the results of previous studies concluded that the content of alisin from the liliaceae tribe, could inhibit the activity of pathogenic bacteria and conversely it was known that the alicin could increase the growth of bacteria that are beneficial to the body's health, because it is often used as a probiotic, namely bacteria *Lactobacillus acidophilus* [5,6]. *Lactobacillus acidophilus* is a living microorganism that can provide beneficial effects for health, if consumed in sufficient quantities [7].

Several studies have shown that probiotics were quite effective for prevention and treatment of various gastrointestinal disorders such as dysentery because use of antibiotics, nosocomial dysentery, dysentery because of bacterial or virus infections [8-10]. Nowadays, the most widely used probiotics are *Lactobacillus casei* Shirota strain, *Bifidobacterium*, and *Lactobacillus acidophilus* [11,12]. The content of alisin in the Liliaceae plant is able to inhibit the growth of pathogenic bacteria and increase the growth of probiotic bacteria, *Lactocabillus acidophilus*. Therefore, it is necessary to conduct research to measure the antimicrobial activity of extracts chive on the growth of *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Lactobacillus acidophilus* bacteria which is one of the probiotic bacteria. So that from the results of this research, in the future the various potential and content of bioactive components contained in extracts chive can be developed and utilized as a superior antimicrobial product of pathogens, improving the quality of food products, can also be developed as materials for making traditional medicine and functional food.

This research also aimed to determine the effect of chive extract to inhibit the growth of pathogenic bacteria and *Lactobacillus acidophilus* and identify the bioactive compounds contained in the chive extract. This research carried out using a factorial completely randomized design, consisting of factor I namely solvent (P) consisting of 3 levels, namely: P$_1$ is water, P$_2$ is methanol, P$_3$ is ethyl acetate. The second factor is concentration (K), chive extract which consists of 4 levels, namely: K$_1$ is 20%, K$_2$ is 40%, K$_3$ is 60%, K$_4$ is 80%.

2. Research Method
The ingredients used were chive, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Lactobacillus acidophilus*, and antibiotic tetracycline. The chemicals and media were used in this research: NA, MHA, physiological NaCl 0.9%, aquadest, oxoid paper, methanol, ethylacetate, dimethylsulfoxide (DMSO), and n-hexane. To testing the chive extract used blank disc paper with a diameter of 6 mm. The disc is inserted into a sterile empty petri dish. Chive extract solution which has been diluted with concentration (%): 20, 40, 60 and 80. Each pipette of 10µl was then dripped on the surface of the disc and waits for 1 hour until the extract solution spreads into the disc. A total of 10 ml of MHA media was poured into sterile petri dishes and allowed to solidify. Sterile cotton swab dipped in culture suspension and gently applied to the surface of the media evenly, then allowed to dry at room temperature for several minutes. Using sterile tweezers, the extracted discs with different concentrations are placed regularly on the surface of the test media. The culture was incubated at an optimum temperature of 37-38°C for 24 hours. After the incubation period, the diameter of the
inhibitory zone (clear area) around the disc is measured using a caliper. The activity of chive extract can be seen with the inhibition zone around the disc. The clear area around the disc paper shows a positive test for inhibition of bacterial growth [14-19]. Flowchart of the research method as shown in Figure 1.

Figure 1. Flowchart of the research method

The research was conducted by using a completely randomized design with two factorials, consisted of: Factor I: solvent type, namely: water, methanol, ethyl acetate, and Factor II: concentration (K) chive extract is 20%, 40%, 60% and 80%. The combination of treatment (PK) is 3 x 4 is 12. This research consisted of 5 stages: 1. preparation of chive extracts, 2. preparation of sample bacteria, 3. examination of chive extracts, 4. analysis of chive powder proximate, 5. phytochemical test.

3. Results and Discussions

3.1 The results of proximate examination

Based on the results of the research conducted, the results of the proximate analysis of chive powder were obtained, as in Table 1. as below:

| Analysis | Chive Powder (%) |
|----------|------------------|
| Water    | 6.80             |
| Ash      | 4.32             |
| Protein  | 9.25             |
| Fat      | 0.62             |
| Carbohydrate | 79.01 |

Table 1. Results of analysis of powdered chive proximate

The results of the chive proximate analysis obtained based on the research were: 9.25% protein content, 0.62% fat content and 79.01% carbohydrate content, this information is expected to be used as a reference for subsequent research for the development of chive powder food products. The result of measuring the water content obtained is less than 10%, which is 6.80%. In sample of chive, the water content that exceeds 10% can be a good medium for fungal growth during storage. Provision of natural, safe and quality food are the major challenge in the food sector. One alternative solution is to reduce the use of chemicals in food formulations and replace them with natural ingredients. The use of natural food additives sourced from plant products that have functional benefits were increasingly developed, such as in food products derived from spices and types of onions.

3.2. The examination result of phytochemical extracts chive

The examination of phytochemical screening of chive extracts were conducted to measure the class of secondary metabolites found in them. The examination results of chive extracts in each solvent showed that the most optimal solvent in taking the active compounds contained in chive extract was ethyl acetate. Ethyl acetate of chive extracts contained alkaloid, flavonoid, glycoside, saponin, and
triterpenoid/steroid compounds, while methanol and water instead of solvents which potentially take the active compounds contained in chive extracts. The chive extract using as a solvent water is known that contained flavonoid compounds, glycosides, and saponins. Methanol extract of chive is known contained of alkaloids, flavonoids, glycosides, and saponins. The results of the phytochemical screening examination of chive extracts were relevant with the results of the examination of antimicrobial activity based on the presence of diameter of the inhibition zone in each solvent. The examination result of phytochemical of chive extracts were using various types of solvents can be seen in Table 2. as below:

Table 2. Phytochemical examination results of chive extracts

| Bioactive Compounds | Solvent chive extracts |
|---------------------|------------------------|
|                     | Water | Methanol | Ethyl acetate |
| Alkaloids           | -     | +        | +            |
| Flavonoids          | +     | +        | +            |
| Glycosides          | +     | +        | +            |
| Saponin             | +     | +        | +            |
| Tanin               | -     | -        | -            |
| Triterpenoid / Steroid | -     | -        | +            |

Description: (+) = Contains a class of compounds  
(-) = Does not contain a class of compounds

Ethyl acetate extract is the most potential extract in inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae* bacteria, relevant with the phytochemical examination results that ethylacetate extracts contained of an active antimicrobial compounds. The active compounds found in extracts chive were alkaloids, these compounds are found in flowering plants, angiosperms (família leguminosae, papavraceae, ranunculaceae, rubiaceae, solanaceae, berberidaceae), and also in monocotyledonous plants (familanaceae and liliaceae). The plant families that contained of alkaloids are liliaceae, solanaceae, and rubiaceae. Unusual plant family that contained alkaloids is papaverae [19]. Bioactive compounds also found in extracts chive are flavonoids. Flavonoids are one of the secondary metabolites produced by a plant that can be found in the leaves, tubers, roots, wood, bark, pollen, flowers and seeds. Some flavonoids in food have certain antihypertensive effects and isoflavones can also function as antifungal, antibacterial, and insecticidal [20]. In addition to flavonoids, there were also glycosides in extract. Glycosides were compounds when hydrolyzed break down into sugar (glycones) and other compounds (aglycones or genin). Distribution of glycosides can be done based on glycones, aglycones, and based on efficacy. Based on its efficacy, glycosides can be classified into cardiac glycosides (cardioactive), antarkinon, saponins, and several other efficacious compounds. Cardiac glycosides are spread in family of apocynaceae, scrophulariaceae, ranunculaceae, and liliaceae. Saponins are divided into two groups, namely sterol saponins (these saponins when hydrolyzed will form sterols) and triterpene saponins (these saponins when hydrolyzed will form triterpenes) [21].

Saponin identification could be done by shaking the extract with warm water in an examination tube and the presence of saponins would be marked in the presence of foam after being shaken and after addition of HCl 2N foam is not lost. The properties of foam saponins are due to the amphiphilic saponin structure, which results in the physical properties of saponins are surfactants. This properties are the same as soaps and detergents. Some examples of saponins include: diosgenin and botogenin from the genus discorcea. Sarmentogenin from the genus strophantus, while sapogenin from the
family liliaceae, amaryllidaceae, and discoreaceae. The next compound found in extracts chive was triterpenoids/steroids. Terpenoids consist of several kinds of compounds, namely monoterpenes, volatile sesquiterpenes, triterpenes, and sterols. Generally, terpenes dissolve in fat and are present in the cytoplasm of plant cells. Usually, terpenes are extracted using ether and chloroform. These compounds are usually identified by the Lieberman-Bouchardat reaction which gives a blackish color. Onion sabrang tuber plants contain almost all phytochemical content, namely: alkaloids, glycosides, flavonoids, steroids and tannins. In this study, bioactive compounds found in extracts chive were: alkaloids, flavonoids, glycosides, saponins and triterpenoids/steroids.

3.3. Antibacterial activity of chive extracts on Escherichia coli

This solvent has an effect on Escherichia coli growth inhibition zones. Each of solvent (water, methanol and ethyl acetate) has a strong inhibitory effect on the growth of Escherichia coli bacteria. The relationship between the effects of solvent type on the inhibition zone diameter of the growth of Escherichia coli bacteria can be seen in Figure 2 as below:

![Figure 2. The effect of extract type and control (+, -) on the inhibition zone of Escherichia coli growth](image)

Description: DMSO = Control (-), Tetracycline = Control (+)

Based on the diagram above, it can be explained that the high and low growth zones of Escherichia coli from extracts chive were caused by the nature of each solvent in taking bioactive compounds found in extracts chive. Bioactive compounds found in extracts chive were: alkaloids, flavonoids, glycosides, saponins and triterpenoids. The flavonoids as the one of the bioactive compounds from chive extracts as antibacterial. The mechanism of action was based on the denaturation of bacterial cell proteins that cause cell death. The results showed that ethylacetate solvent had the largest inhibitory zone compared to methanol and water solvents, this was due to the properties of semi polar ethylacetate, it could take all of active compounds in polar and nonpolar extracts chive, while methanol and water solvents only able to take polar bioactive compounds. In this study, DMSO was used as a negative control and tetracycline as a positive control. The diameter of tetracycline inhibition zone on the sample bacteria was 11.5 mm.

Tetracycline with a concentration of 30 μg/ml gave a strong inhibition to the growth of Escherichia coli. Antimicrobial of a sample material was called strong inhibitory, if it has a inhibitory zone greater than 11 mm, medium inhibitory with 6-11 mm inhibition zone, and if the inhibitory zone smaller than 6 mm is called weak / low inhibition. If compared with the extracts chive in solvents and their respective concentrations, showed that tetracycline antibiotics had a lower diameter of the
inhibition zone. It was because the secondary metabolite compounds contained in chive extract have a higher inhibitory ability compared to tetracycline. Tetracycline is an antibiotic that works bacteriostatically. The inhibition of protein synthesis will cause loss of stiffness and strength of the cell wall, so that bacteria will experience death. Based on the results of the study it was found that DMSO did not provide inhibition toward the sample bacteria. It proved that DMSO does not have crucial role in inhibiting the growth of bacteria tested.

3.4. Antibacterial activity of chive extracts on Staphylococcus aureus

The relationship between the effects of solvent types on the diameter of Staphylococcus aureus growth inhibition zones can be seen in Figure 3. as below:

Figure 3. The effect of extract type and control (+, -) on the growth inhibition zone of Staphylococcus aureus

Based on the figure above, it was known that the type of solvent used in chive extracts affected the diameter of the growth inhibition zone of Staphylococcus aureus bacteria, due to the different properties of each solvent in taking bioactive compounds found in chive extracts. The difference in the components extracted in the ethyl acetate methanol and water solvents were steroids and alkaloids. The content of steroid compounds in ethylacetate diffuses more easily and was able to inhibit bacterial growth. Steroids had nonpolar properties to semipolar. Staphylococcus aureus is a gram-positive bacteria, this bacteria grows easily on a variety of media, metabolizes actively by fermenting carbohydrates and produces various pigments ranging from white to dark yellow pigments. S. aureus for yellow colonies and S. albus for white colonies.

The factor that influence the occurrence of inhibitory zones were the ability to diffuse antimicrobial material into the media and their interaction with the tested microbes, the number of microbes tested, the microbial growth rate of the test and the level of microbial sensitivity to antimicrobial material, affecting the diameter of the inhibitory zone of bacterial growth. The diameter of tetracycline antibiotic inhibition zone in Staphylococcus aureus bacteria was classified as effective at 10.5 mm. Secondary metabolite compounds contained in chive extract with water solvents had a smaller inhibitory ability compared to tetracycline. In the ribosome, tetracycline binds to the 30S subunit and blocks the incorporation of amino acids into the peptide chain and causes protein
synthesis to be inhibited. The inhibition of protein synthesis would cause the loss of stiffness and strength of the cell, so that bacteria would death.

3.5. Antibacterial activity of chive extracts on *Shigella dysenteriae*

Based on the results of the study, it was known that each type of solvent has different inhibitory activity on the growth of *Shigella dysenteriae* as shown in Figure 4.

![Inhibition Zone Diameter](image)

Description: DMSO = Control (-), Tetracycline = Control (+)

**Figure 4.** The effect of extract type and control (+, -) on the inhibition zone of *Shigella dysenteriae* growth

The diameter of the highest inhibitory zone was obtained in ethylacetate solvent, while the lowest was obtained in water solvent. This type of solvent affected the diameter of the *Shigella dysenteriae* growth inhibition zone. The content of antimicrobial compounds found in chive extract can inhibit the growth of *Shigella dysenteriae* bacteria. Diameter of tetracycline inhibitory zone for growth inhibition of *Shigella dysenteriae*, which was 10.5 mm. Tetracyclines provides an active inhibition against the growth of *Shigella dysenteriae*. Tetracycline antibiotics using a negative controls, whereas DMSO using as a positive control. Based on the results of the study, it was found that DMSO did not provide inhibition on the four bacteria examined. It proved that DMSO does not have crucial role in inhibiting the growth of bacteria examined.

3.6. Antibacterial activity of chive extracts on *Lactobacillus acidophilus*

Based on the results of the study, it was found that the type of solvent and the concentration of chive extract did not inhibit the growth of *Lactobacillus acidophilus*. These bacteria can still grow after testing the chive extract by using water, methanol, and ethylacetate. Previous research reported that alicin inhibits differently between the intestinal microflora which is beneficial with harmful intestinal bacteria. Antibacterial activity was observed based on the inhibition zone diameter in *Escherichia coli* bacteria, with inhibitory power 10 times better than the *Lactobacillus casei* for the same concentration of chive. The content of oligosaccharide compounds in chive extract was a good growth medium for *Lactobacillus acidophilus*. Generally, tubers contained high amounts of raffinose oligosaccharides. Insoluble oligosaccharides such as raffinose, fructo oligosaccharides, galactosyl-lactose, isomalt o oligosaccharides or transgalacto-siloligo saccharides, have been known to increase the number of indigenous bifidobacteria and other lactic acid bacteria. *Lactobacillus acidophilus* bacteria were bacteria that can be used for probiotic food products. Several studies have shown that probiotics were quite effective for prevention and treatment of various gastrointestinal disorders such as dysentery of antibiotic use, nosocomial dysentery, and dysentery of bacterial infections. Based on the results of this study, it is known that, chive extract is one of the good media for the growth of *Lactobacillus*...
*acidophilus* bacteria, so the chive extract is very potential for the development of food products, especially those related to probiotics.

3.7. The data of antibacterial activity examination of chive extract on each solvent against sample bacteria

Overall, the results of this study were examining chive extract on the growth of *Escherichia coli* sample bacteria, *Staphylococcus aureus*, and *Shigella dysenteriae* that showed the growth inhibition activity (inhibitory zone). The magnitude of the microbial growth inhibition zone examined by the extract was seen as a clear area (clear zone) around the disc (the backing paper) contained the extract. The ability of extracts to inhibit microbial growth was caused by the presence of active compounds contained in extract chive. These compounds act as active ingredients that could inhibit the growth of bacteria sample. In addition to the ability of these compounds, there were major components of the genus *Allium* including chive plants, namely sulfur compounds. The relationship between the effects of solvent types on the zones of growth inhibition of *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Lactobacillus acidophilus* can be seen in Figure 5.

The difference in diameter of the inhibition zone in each solvent was caused by the polarity of each solvent. In this study, nonpolar solvents were taking the more active compounds that found in extract chive. The ability of ethylacetate solvent to take the more active compounds from methanol and water solvents, because the ethyl acetate solvent was semi polar, so it can take both polar and nonpolar compounds. In addition to solvent types, the concentration of each chive extract also affected the inhibition zone diameter of *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and
Lactobacillus acidophilus. The results of the antimicrobial activity of chive extract showed that the higher the extract concentration, the greater the diameter of the inhibitory zone.

4. Conclusion
Based on the results is concluded that the ethylacetate extract of chive contains alkaloids, flavonoids, glycosides, saponins, and triterpenoids/steroids, while the water extract of chive contains flavonoids, glycosides, and saponins, and extracts of methanol contains alkaloids, flavonoids, glycosides, and saponins. The type of solvent and solvent concentration gave a very significant effect on the growth inhibition of Escherichia coli, Staphylococcus aureus and Shigella dysenteriae. The interaction between types and solvents concentration had no significant effect on the growth inhibition of Escherichia coli, Staphylococcus aureus, and Shigella dysenteriae. Chive extract with ethyl acetate solvent was the most effective extract in inhibiting the growth of Escherichia coli, Staphylococcus aureus, and Shigella dysenteriae, and does not give inhibitory power to the growth of Lactobacillus acidophilus. Based on the results of this study, it is known that chive extract is a good medium for the growth of Lactobacillus acidophilus bacteria, so that chive extract is very potential for the development of food products, especially probiotic food.

References
[1] Shah N C 2014 Status of cultivated and wild allium species in India The Scitech Journal ISSN 2347-7318 ISSN 2348-2311
[2] Matthew R V and Budoff J 2016 Garlic and heart disease The Journal of Nutrition Volume 146 Issue 2 Pages 4168–4215
[3] Dadile M A 2017 Antibacterial potency of garlic extract against certain skin pathogenic bacteria Novel Research in Microbiology Journal (2017) 1(1) 3-13
[4] Shinkafai S A 2013 Antibacterial activity of Allium Cepa (onion) on some pathogenic bacteria associated with ocular infections Scholars Journal of Applied Medical Sciences 147-151
[5] Beena Uppal 2015 A comparative study of bacterial and parasitic intestinal infections in india Journal of Clinical and Diagnostic Research ISSN-0973-709x
[6] Jain P and Sharma P 2012 Probiotics and their efficacy in improving oral health Journal of Applied Pharmaceutical Science Vol 2 (11) pp 151-163
[7] Brady L J, Gallher D and Busta F 1998 The role of probiotic cultures in the prevention of the colon cancer J Nutrition 41(1) 50-61
[8] Markowitz J E and Bengmark S 2003 Probiotics in health and disease in the pediatric patient Pediatric Clin North Am 3(1) 49-54
[9] Samarzua D 2009 Probiotic bacteria in prevention and treatment of diarrhea Mljekarstvo 59 (1) 28-32
[10] Cameron D 2017 Probiotics for gastrointestinal disorders proposed recommendations for children of the Asia Pacific region World J Gastroenterol 23(45) 7952-7964
[11] Duggan C, Walker W A and Watkins J B 2003 Nutrition In Pediatrics 3rd Edition London BC Decker 3(1) 223-233
[12] Kerrya R G 2018 Benefaction of probiotics for human health A review Journal of Food and Drug Analysis Volume 26 Issue 3 July 2018 Pages 927-939
[13] Yuli R S 2014 The comparison of extraction method and solvent variation on yield and antioxidant activity of brassica oleracea L var capitata f rubra Extract Trad Med J Vol 19(1) p 43-48
[14] Azwanaida N 2015 a review on the extraction methods use in medicinal plants azwanaida Med Aromat Plants 4:3
[15] AOAC 1995 Official Methods of Analysis of The Association of Analytical Chemists Washington D C p 1-71
[16] Depkes RI 1995 Farmakope Indonesia Edisi IV Jakarta Departemen Kesehatan Republik Indonesia hlm 267-271
[17] Whitmore B and Naidu A S 2000 Thiosulfinatges Natural Food Antimicrobial System CRC Press. New York
[18] Farnsworth N R 2006 Biological and phytochemical screening of plants Journal of Pharmaceutical Sciences 55(3) 257-259
[19] Strika I 2017 Antimicrobial effects of garlic (Allium sativum L) Cardiff University Bulletin of the Chemists and Technologists of Bosnia and Herzegovina ISSN 0367-4444 p 17-20
[20] Prima S O 2006 Isolation and identification of flavonoid compound extractire ethyl acetate fraction extracted from the rhizomes fingerroot of (Boesenberia pandurata (Roxb.) Schlecht (Zingiberaceae) Indo J Chem 2006 6 (2) 219-223
[21] Steinegger E and Hansel R 2010 Pharmacological properties and analysis Current Pharmaceutical Analysis 5(1) 47-68
[22] Alorainy M S 2011 Evaluation of antimicrobial activity of garlic (Allium sativum) against Escherichia coli Journal of Agricultural and Veterinary Sciences 4(2) 149-157
[23] Zul A 2012 Effect of addition pliek U in feed on histomorphometric of small intestine villi of broiler The Proceedings of The 2nd Annual International Conference Syiah Kuala University and The 8th IMT-GT Uninet Biosciences Conference Volume 2 Number 1
[24] Connors K P 2013 Optimizing antibiotic pharmacodynamics for clinical practice Pharmaceut Anal Acta 4:3 DOI 10.4172/2153-2435 1000214
[25] Khan S U 2018 antimicrobial potentials of medicinal plant’s extract and their derivedsilver nanoparticles A focus on honey bee pathogen Saudi Journal of Biological Sciences p 17-26

Acknowledgement
The author would express her gratitude to Rector of Universitas Katolik Santo Thomas, Dean of Faculty of Agriculture Universitas Katolik Santo Thomas, for facilitating the research and the presentation at ICAFAT 2018.