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FATTY ACID COMPOSITION AND ANTIBACTERIAL ACTIVITY OF CLOVE (*Syzygium aromaticum*) AND CARROT (*Daucus carota*) AS CANDIDATE OF ADDITIVE FOR BROILER CHICKENS

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Abstract

This study aimed to evaluate the fatty acids and to determine the antibacterial activity of clove (Syzygium aromaticum) and carrot (Daucus carota) against the pathogen Escherichia coli and Staphylococcus aureus. Clove and carrot were prepared from fresh plants in three concentrations (2.5%, 5% and 10%), the positive control of chloramphenicol and the negative control of DMSO. Disc diffusion method has been used to determine the antimicrobial activities of clove and carrot. The results showed that dominant fatty acids in flower of clove were Linoleic acid, Palmitic acid, Cis-13,16-Docosadienoic acid, Stearic acid, Oleic acid, Linolenic acid, γ-Linolenic acid, and fatty acid total was 9.59% w/w, whereas fat content of clove was 4.19 %w/w. Moreover, in carrot, Linoleic acid was the major component followed by Palmitic acid, Linolenic acid, Oleic acid, Stearic acid, γ-Linolenic acid, and fatty acid total was 37.65% w/w, whereas fat content of carrot was 2.21% w/w. Inhibition zone of clove for E. coli at concentration between 2.5%, 5%, and 10% showed the same response (6 mm),and was lower than that of positive control chloramphenicol (7.69 mm), however, for S. aureus inhibition zone at concentration 10% (8.64 mm) was almost the same with positive control (10.52 mm). Inhibition zone of carrot for E. coli at concentration 2.5% was the same with positive control (9.75 mm), moreover, for S. aureus at concentration 10% was lower than positive control but still proved effective against bacteria. It can be concluded that clove flower was better as natural antimicrobials for E. coli and carrot was for S. aureus. So, clove and carrot can be used as feed additives in broiler diet

Key words*: additive, antibacterial, carrot, clove, fatty acid.*

INTRODUCTION

Recent ban on the use of antibiotic growth promoters (AGP) in poultry feeds has drawn the concerns of researchers towards the presence of natural substances like medicinal herbs as a new class of additives to animal and poultry feeds. There are plenty resources of different kinds of medicinal herbs which can be used as alternative feed additives for poultry (Vinus et al., 2018). Herbs and their mixture can ameliorate the performance of birds by improving digestive tract function by anti-inflammatory, anti-oxidative and anti-microbial effects. Herbs may exert multiple functions in the bird's body system (Hernandez et al., 2004) It has been vivid that the potential of medicinal herbs as the valuable source of therapeutics aids has attained a global significant place in the health system all over the world not only for humans but also for animals as well as birds (Dhama et al., 2015). Antibiotic and antibacterial medications still used in poultry industry in several indications including therapeutic treatment, prevention or as traditional growth promoters (Diarra and Malouin, 2014). Particular attention is drawn to two classes of antimicrobial lipid, namely fatty acids (hydrocarbon chains with a carboxylic acid functional group) and monoglycerides (esterified of a fatty acid and glycerol molecule). Fatty acids are released from lipids, typically by the action of enzymes, to become free fatty acids, which have vast and potent biological activities (Desbois and Smith, 2010). The biological activities of free fatty acids have roles in host defences against potential opportunistic or pathogenic microorganisms. An important aspect of this is growth inhibition or quick destroying of bacteria. Several studies for understanding the mechanism of the anti-bacterial effects of fatty acids from biological sources such as algae, animals and plants have been done by several researchers (Wille and Kydonieus, 2003; Desbois and Smith, 2010). Several researchers investigated the antimi-

crobial activity of fatty acid derivatives. However, they show poor antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* (Shukla et al., 2018; Walvekar et al., 2018).

Many fatty acids, such as lauric, palmitic, linolenic, linoleic, oleic, stearic, and myristic acids are known to have antibacterial and antifungal properties (Seidel and Taylor, 2004). Galbraith et al. (1971) reported that lauric acid is the most potential gram-positive antibacterial agent among the saturated fatty acids, while linoleic acid is the most potential gram-positive antibacterial agent among the unsaturated fatty acids. Yoon et al. (2018) reported that oleic acid shows antibacterial activity against *S. aureus* through damaging the bacterial cell membrane.

The antibacterial activity of long-chain unsaturated fatty acids has been well known for many years. These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active (Sun et al., 2003). However, their primary molecular target still remains unknown (Zheng et al., 2005).

Fatty acids with antibacterial activity have been isolated from several plants. Cerdeiras et al. (2000) identified 11-O-(6'-O-acetyl-β-D-glucopyranosyl)-stearic acid as the main antibacterial component of aerial parts of *Ibicella lutea*. This fatty acid derivative showed an interesting antibacterial activity, being active against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *S. aureus* with the Minimal Inhibitory Concentration value of 9 $\mu g ml^{-1}$ against *S. aureus*. Dilika et al. (2000) described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum*. Linoleic and oleic acids inhibited the growth of Gram-positive: *B. subtilis*, *Micrococcus kristinae* and *S. aureus*, and linoleic acid also showed activity against *B. cereus* and *B. pumilis*. Both acids displayed no activity against Gram-negative: *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Serratia marcescens*.

Bacteria *S. aureus* and *E. coli* are the bacteria that cause most infections in the community and nosocomial infections. *S. aureus* is a major pathogen in humans. *S. aureus* is positive coagulase, which distinguishes it from other species. Nearly everyone has experienced a variety of *S. aureus* infections during his lifetime, from severe food poisoning or small skin infections, to infections that cannot be cured (Brooks et al., 2008).

The *E. coli* bacteria are Gram-negative bacteria, while *S. aureus* bacteria are Gram-positive bacteria. The antibacterial agent against the growth of test bacteria has a mechanism of antibacterial activity that is thought to attack various parts of bacterial cells, namely cell walls, cell membranes, cell proteins, or bacterial cell nucleic acids. Besides these antibacterial substances are also able to inhibit bacterial cell metabolism (Gan et al., 1995; Pelczar et al., 2010).

Gram-positive bacteria represent the causative agents of both animal intestinal diseases and potentially lethal foodborne diseases in humans. *Listeria monocytogenes* and *S. aureus* are considered to be the widespread pathogens causing serious illnesses and systematic disorder both in animals and humans (McLauchlin and Rees, 2009). Phytogenic additives, including organic acid, present an alternative as they enhance a number of important processes in the animal body as well as they can be used also in the food industry because of their antibacterial properties (Karaskova et al., 2015).

The primary and secondary habitats of *E. coli* are the intestinal tract of warm-blooded animals and the environment, respectively. In poultry, as in humans, *E. coli* resides in the lower digestive tract, which it colonizes in the first 24 h after hatching or birth (Ballou et al., 2016; Stromberg et al., 2017). Ohta et al. (1995) demonstrated the antibacterial activity of α-linolenic acid against methicillin-resistant *S. aureus* (MRSA). McDonald et al. (1981) reported on the susceptibility of several strains of MRSA to linolenic acid and hydrolysed linseed oil (containing 52% linolenic acid in the ester form). Yoon et al. (2018) reported that all tested Gram + sp. was susceptible to treatment with 0.01 mM arachidonic acid C20:4. Bactericidal effect of arachidonic acid treatment on *S. aureus* depended on treatment time and drug concentration.

Clove (*Syzygium aromaticum*) is the aromatic flower buds of a tree in the family Myrtaceae. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. Clove plants have special characteristics, because all parts starting from the roots, stems, leaves, to the flowers, contain organic acids or essential oils. Cloves has been used as an antimicrobial (Valero and Salmeron, 2003).

Many vegetable plants can be used as medicinal plants, one of which is carrots (*Daucus carota* L.). Carrots are vegetable plants that have many uses for public health services in the world. Besides being rich in nutritional content, especially vitamin A is also efficacious for healing various diseases (Rukmana, 1995). Carrot (*Daucus carota* subsp. *sativus*) is a root vegetable, usually orange in colour, though purple, black, red, white, and yellow cultivars exist. They are a domesticated form of the wild carrot, *Daucus carota*, native to Europe and Southwestern Asia. The plant probably originated in Persia and was originally cultivated for its leaves and seeds. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines. The roots contain high quantities of alpha- and beta-carotene, and are a good source of vitamin K and vitamin B6.

Fatty acids are widely occurring in natural fats and dietary oils and they are known to have antibacterial and antifungal properties. However, little is known on antibacterial activity of clove and carrot meal, and this study for the first time determines the fatty acid composition and the antibacterial activity potency of fatty acids in clove and carrot.

MATERIALS AND METHODS

The fatty acid analysis was carried out by A.O.A.C. Official Methods 2012.13: 991.33 (fatty acid in oils and fats; preparation methyl ester). And analysis of fat content according to A.O.A.C 2012: 991.36.

The dried clove flower and freshly carrot used in the study was collected from the local market in Manado, North Sulawesi of Indonesia. Carrot sample was shade dried, and then, dried flower clove and carrot were powdered.

Fat and fatty acid were extracted from clove and carrot by hydrolytic method. Fat was extracted into ether, then methylated to fatty acid methylesters (FAMEs). FAMEs then quantitatively measured by gas chromatography. The FAME components were identified using the retention time established using reference standard for FAs and percentage of individual FAME was made in relation to total area of the chromatogram.

Antibacterial tests were carried out by disc diffusion method (Pal et al., 2007). *S. aureus and E. coli* test bacteria were rejuvenated first, then a bacterial suspension was made. The clove and carrot meal were each made with a concentration solution 2.5%, 5%, and 10% using DMSO solvents. As much as 0.3 mL of bacterial suspension was put into a Petri dish then 15 mL of NA media was added to the Petri dish, homogenized and then condensed. The sterile 6 mm filter paper discs (Whatman No. 3) were impregnated with the 2.5%, 5%, and 10%/disc stock solution of clove and carrot, and placed on the inoculated agar. Negative controls were prepared using DMSO. DMSO solvent is used in this study because DMSO can dissolve polar and nonpolar compounds and DMSO will not interfere with observations because it does not provide activity on bacterial and fungal growth. Chloramphenicol (0.1%/disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. Chloramphenicol is used as a positive control for bacteria because it belongs to a broad spectrum of antibiotics that can inhibit the growth of Gram-positive and Gram-negative (Octaviani et al., 2019).

The inoculated plates were incubated at 37^0C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. All inhibitory tests were performed in triplicate. Growth bacteria were observed, and the zone of inhibition was calculated in millimetres carefully.

RESULTS AND DISCUSSIONS

Fatty acid composition and antibacterial activity of clove (*Syzygium aromaticum*) and carrot (*Daucus carota* L.) as candidate of additive for broiler chickens have shown in Tables 1 and 2. Result showed that dominant

fatty acids in flower of clove were Linoleic acid (C18:2n6c), Palmitic acid (C16:0), Cis-13,16-Docosadienoic acid (C22:2), Stearic acid (C18:0), Oleic acid (C18:1n9c), Linolenic acid (C18:3n3), γ-Linolenic acid (C18:3n6), and fatty acid total was 9.59% w/w, whereas fat content of clove was 4.19% w/w. Moreover, in carrot, Linoleic acid (C18:2n6c) was the major component followed by Palmitic acid (C16:0), Linolenic acid (C18:3n3), Oleic acid (C18:1), Stearic acid (C18:0), γ-Linolenic acid (C18:3n6), and fatty acid total was 37.65% w/w, whereas fat content of carrot was 2.21% w/w. The flower of *Syzygium aromaticum* and root of *Daucus carota* L showed an almost similar fatty acid profile.

Table 1. Fatty acid of clove and carrot

Parameter	Result	
	Clove (% w/w)	Carrot (% w/w)
Fat Content	4.19	2.21
Fatty acid		
Caprilic acid, C8:0	0.06	-
Capric acid, C10:0	0.02	-
Lauric acid, C12:0	0.11	0.04
Tridecanoic acid, C13:0	0.11	-
Myristic acid, C14:0	0.20	0.08
Pentadecanoic acid, C15:0	0.02	0.13
Palmitic acid, C16:0	1.63	4.62
Palmitoleic acid, C16:1	0.06	0.15
Heptadecanoic acid, C17:0	0.06	0.16
Cis-10-Heptadecanoic acid, C17:1	0.04	0.14
Stearic acid, C18:0	0.65	0.87
Elaidic acid, C18:1n9t	-	0.07
Oleic acid, C18:1n9c	0.62	1.49
Linoleic acid, C18:2n6c	2.72	25.41
Arachidic acid, C20:0	0.31	0.36
γ-Linolenic acid, C18:3n6	0.39	0.75
Cis-11-Eicosenoic acid, C20:1	0.07	0.10
Linolenic acid, C18:3n3	0.52	2.74
Heneicosanoic acid, C21:0	0.02	0.06
Cis-11,14-Eicosedienoic acid, C20:2	-	0.03
Behenic acid, C22:0	0.21	0.23
Arachidonic acid, C20:4n6	-	0.03
Cis-13,16-Docosadienoic acid, C22:2	1.28	-
Tricosanoic acid C23:0	-	0.09
Lignoceric acid, C24:0	0.51	0.13
Fatty acid – total	9.59	37.65

Inhibition zone of clove for *E. coli* at concentration between 2.5%, 5%, and 10% showed the same response (6 mm), and was lower than that of positive control chloramphenicol (7.69 mm), however, for *S. aureus* inhibition zone at concentration 10% (8.64 mm) was almost the same with positive control (10.52 mm). Inhibition zone of carrot for *E. coli* at concentration 2.5% was the same with positive control (9.75 mm), moreover, for *S. aureus* at concentration 10% was lower than positive control but still proof effective against Gram-positive bacteria.

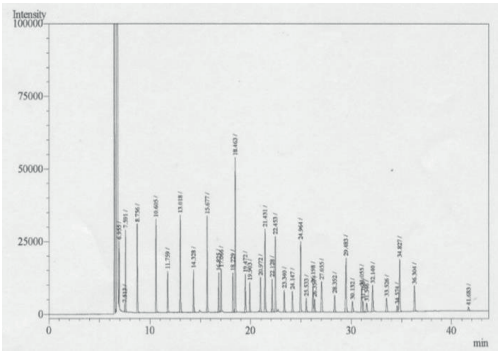

Figure 1. Graphic of Standard of FAME

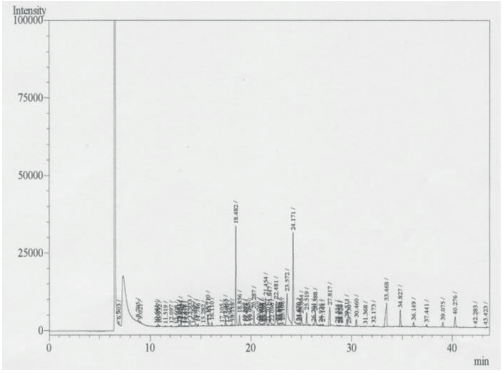

Figure 2. Graphic of Clove Fatty Acids

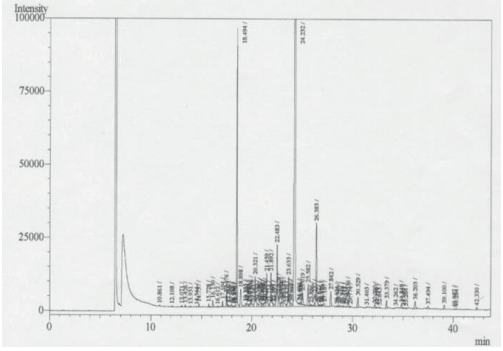

Figure 3. Graphic of Carrot Fatty Acids

Clove demonstrated the highest zone of inhibition at 10% concentration on *S. aureus* 8.64 mm and the lowest inhibition on *E. coli* with a diameter 6 mm. The clove generally proved effective against Gram- positive bacteria used in this study even at the highest concentration while other Gram-negative organisms showed resistance at the lowest concentration. On the other hand, carrot demonstrated the highest zone of inhibition at lowest concentration (2.5%) on *E. coli* but showed highest zone of inhibition at highest concentration (10%) on *S. aureus*. This study recommended the use of this clove in combating some of *E. coli* that are resistance.

Overall it can be seen for clove inhibition on *S. aureus* that the higher the concentration of the compound given, the greater the diameter of the inhibitory region formed. This is consistent with the theory put forward by Pelczar and Chan (1988), that the greater the concentration of the antimicrobial compounds tested, the greater the antimicrobial activity of the compound.

Table 2. Diameter zone of inhibition of clove and carrot for bacterial

Sample	Concentration (%)	Zone of inhibition (mm)	
		E. coli	*S. aureus*
Clove	2.5	6.00	6.00
	5	6.00	6.00
	10	6.00	8.64
Chloramphenicol (C+)	0.1	7.69	10.52
DMSO (C-)		0	0
Carrot	2.5	9.75	6.00
	5	6.00	6.65
	10	7.2	7.96
Chloramphenicol (C+)	0.1	9.75	12.38
DMSO (C-)		0	0

Compared to Huda et al. (2018) reported that the clove flower extract of 10% to 100% concentration was able to inhibit the growth of *S. aureus* bacteria. The lowest concentration of clove flower extract which can inhibit the concentration of 10% with average of 15.87 mm. and highest concentration of clove flower extract 100% concentration obtained average 21.40. While the clove flower extract at 70% concentration with mean of 19.16 mm has effectively inhibited bacterial growth when compared with positive control of Amikacin antibiotics with mean of 18.8 mm. The essential oils in clove flower showed the largest antibacterial activity which was about 25.85-26.75 mm, while in the flower stalks and clove leaves showed an activity with inhibition zone of 20.60-21.20 mm and 18.04-18.58 mm, respectively (Lova et al., 2018). In our study, we used clove meal for against the bacteria, so, maybe we have to add the level of concentration of clove to increase the inhibition zone.

Compared to Sirait et al. (2016) reported that extract ethanol 96% of carrot (*Daucus carota* L.) root in 5%, 10%, 20%, 40%, and 80% concentrations have to provide activities that inhibits the growth of test bacteria. Any increase in concentrations of 5% (3.50 mm), 10% (3.67 mm), 20% (4.83 mm), 40% (5.16 mm) and 80% (6.67 mm) for a review of *E. coli* and a concentration of 5% (3.17 mm), 10% (3.83 mm), 20% (4.00 mm), 40% (4.17 mm) and 80% (4.33 mm) to review the bacterium *S. aureus*. In this study, any increase in concentration of carrot meal maybe will increasing the inhibition zone.

Gonelimali et al. (2018) reported that plant extracts significantly affected the cell membrane of Gram-positive and Gram-negative bacteria. Plant extracts: roselle (*Hibiscus sabdariffa*), rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), and thyme (*Thymus vulgaris*) are of great value as natural antimicrobials and can use safely as food preservatives.

The antimicrobial properties of fatty acids are well known and there is a close relationship between the structure of fatty acids and their ability to function as antimicrobial agents. Saturated fatty acids are effective against microorganisms at lower chain lengths, while monounsaturated and polyunsaturated fatty acids with longer chain lengths are more effective. The position of double bonds is significant for long chain fatty acids (McGaw et al., 2002). Anzaku et al. (2017) reported that free fatty acids (FFA) of various chain lengths (C8-C18) have antibacterial activity against a range of Gram-positive bacteria, but not against a number of Gram-negative bacteria. In this study, carrot have high Linoleic acid, so it is effective against *E. coli* compared to clove.

Clavijo and Flo´rez (2018) reported that the extraction of energy and nutrients from food

requires interaction between the biochemical functions of the chicken and the microbiota present in the gastrointestinal tract. Thus, the selection of beneficial microbiota plays an important role in the production, health, protection from pathogens, detoxification, and modulation of the immune system. The presence of pathogenic bacteria in the broiler chicken microbiota is important to animal and human health alike. Among the taxa that can cause illness in humans and that have been reported in the chicken microbiota are *Campylobacter* (principally *Campylobacter jejuni* and *Campylobacter coli*), *Salmonella enterica*, *Escherichia coli*, and *Clostridium perfringens* (Oakley et al., 2014).

In poultry, *E. coli* infections include egg peritonitis, omphalitis, coligranuloma, swollen head syndrome, cellulitis, and colisepticaemia. Colisepticaemia is a severe systemic form of infection. Omphalitis is a major factor responsible for early chick mortality during the first few days after hatching (EL-Sawah et al., 2018). In this study, clove and carrot were not unconventional feed for poultry, moreover, it relating to its part of feed supplement.

CONCLUSIONS

From the results, that these plants contains high percentage of linoleic and significant amount of palmitic acids. Plants showed the power of antibacterial activity. It can be concluded that clove flower was better as natural antimicrobials for *E. coli* and carrot was for *S. aureus*. However, further work needs to be carried on characterization of fatty acid compounds and test that fatty acids potential for antibacterial activity.

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REFERENCES

Anzaku, A. A., Akyala, J. I., Juliet, A., Obianuju, E. C. (2017). Antibacterial activity of lauric acid on some selected clinical isolates. *Ann. of Clinic. and Lab. Res.,* 5(2), 170–174.

Ballou, A. L., Ali, R. A., Mendoza, M. A., Ellis, J. C., Hassan, H. M., Croom, W. J. (2016). Development of the chick microbiome: how early exposure influences future microbial diversity. *Front Vet*. Sci., 3, 2.

Cerdeiras, M. P., Fernández, J., Soubes, M., Vero, S., Ferreira, F., Moyna, P., Olano, I., Vázquez, A. (2000). A new antibacterial compound from *Ibicella lutea*. *J. of Ethnopharm*. 73, 521–525.

Clavijo. V., Flo´rez, M. J. V. (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poult. Sci*., 97, 1006–1021.

Dilika, F., Bremner, P. D., Meyer, J. J. M. (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia*, 71, 450–452.

Desbois, A. P., Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Appl. Microbiol. Biotechnol*., 85, 1629–1635.

Diarra, M. S., Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Front Microb*., 5, 282. doi:10.3389/fmicb.2014.00282.

Dhama, K., Latheef, S. K., Mani, S., Samad, H. A., Karthik, K., Tiwari, R., Khan, R. U., Alagawany, M., Farag, M. R., Aiam, G. M., V. Laudadio, V., Tufarelli, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production-A Review. *Int. J. of Pharm*., 11(3), 152-176.

EL-Sawah, A. A., Dahshan, A. H. M., El-Nahass, El-S., El-Mawgoud, A. I. A. (2018). Pathogenicity of Escherichia coli O157 in commercial broiler chickens. Beni-Suef Univ. *J. of Basic and Appl. Sci*., 7, 620–625.

Galbraith, H., Miller, T., Paton, A., Thompson, J. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Microbiol*., 34, 803-813.

Gibbons, S. (2004). Anti-staphylococcal plant natural products. *Natural Product Reports*, 2, 263-277.

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. S. R. (2018). antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microb*., 9, 1-9.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., Megias, M. D. (2004). Influence of tow plant extracts on broiler performance, digestibility, and digestive organ size. *Poult. Sci*., 83, 169-174.

Huda, M., Rodhiansyah, Ningsih, D. S. (2018). Efektivitas ekstrak bunga cengkeh (*Eugenia aromatic*) terhadap pertumbuhan bakteri *Staphylococcus aureus*. *Jurusan Analis Kesehatan,* 7(1), 710-716.

Kammon, A., Almaeyoufi,A., Asheg, A. (2019). In vitro antimicrobial activity of clove oil *in vitro* against gram negative bacteria isolated from chickens.

Approches in Poult., Dairy and Vet. Sci., 6(2), 542-456.

Karaskova, K., Suchy, P., Strakova E. (2015). Current use of phytogenic feed additives in animal nutrition: A review. *Czech J. of Anim. Sci.,* 60, 521-530.

Lova, I. P. S. T., Wijaya, W. A., Paramita, N. L. P. V., Putra, A. A. R. Y. (2018). Perbandingan uji aktivitas antibakteri minyak atsiri daun, tangkai bunga dan bunga cengkeh bali (*Syzygium aromaticum* L.) terhadap bakteri *Propionibacterium acne* dengan metode difusi disk. *J. Kimia*, 12(1), 30-35.

McDonald, M. I., Graham, I., Harvey, K. J., Sinclair, A. (1981). Antibacterial activity of hydrolysed linseed oil and linolenic acid against methicillin-resistant *Staphylococcus aureus*. *The Lancet*., 2, 1056.

McGaw, L. J., Jäger, A. K., Van Staden, J. (2002). Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia*, 73, 431-433.

McGaw, L. J., Jäger, A. K., van Staden, J. (2002). Antibacterial effects of fatty acids and related compounds from plants. *South African J. of Botany*, 68, 417-423.

McLauchlin J., Rees, C. E. (2009). *Genus Listeria*. In: Whitman W.B. (Ed.): Bergey's Manual of Systematic Bacteriology, Vol. 3. New York, USA: Springer-Verlag Publishing House, 244-257.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., Lee, M. D., Collett, S. R., Johnson, T. J., Cox, N. A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiol. Lett*., 360, 100–112.

Ohta, S., Y. Shiomi, A. Kawashima, O. Aozasa, T. Nakao, T. Nagate, K. Kitamura, H. Miyata (1995). Antibiotic effect of linolenic acid from Chlorococcum strain HS-101 and *Dunaliella primolecta* on methicillin-resistant *Staphylococcus aureus*. *J. of Appl. Phycology*, 7, 121-127.

Pal, S., Kyung, T. Y., Myong, S. J. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol*., 73(6), 1712-1720.

Rukmana, R., Sugandi, S. 1995. *Hama Tanaman dan Teknik Pengendalian*. Bumi Aksara. Jakarta.

Seidel, V., Taylor, T. P. W. (2004). In vitro activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. *Int. J. Antimicrob. Agents*, 23, 613-619.

Shukla, S., Hegde, S., Kumar, A., Chaudhary, G., Tewari, S. K., Upreti, D. K., Pal, M. (2018). Fatty acid composition and antibacterial potential of Cassia tora (leaves and stem) collected from different geographic areas of India. *J. of Food and Drug Anal*., 26, 107-111.

Sirait, A. Y., Pelealu, N. C., Yamlean, P. V. Y. (2016). Uji daya antibakteri ekstrak etanol umbi wortel (*Daucus carota* L.) terhadap *Staphylococcus aureus* dan *Escherichia coli* secara *in vitro*. *Pharmacon J. Ilmiah Farmasi*, 5(4), 145-154.

Sun, C. Q., O'Connor, C. J., and Roberton A. M. (2003). Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. *FEMS Immunol. Med. Microbiol*., 36, 9-17.

Valero, M., Salmeron, M. C. (2003). Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *Int. J. Food Microbiol*., 85, 73-81.

Wille, J. J., Kydonieus, A. (2003). Palmitoleic acid isomer (C16:16) in human skin sebum is effective against Gram-positive bacteria. *Skin Pharmacol Appl. Skin Physiol*., 16, 76-87.

Yoon, B. K., Jackman, J. A., Valle-González, E. R., Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int. J. Mol. Sci*., 19(1114), 1-40.

Zheng, C. J., Yoo, J. S., Lee, T. G., Cho, H. Y., Kim, Y. H., Kim, W. G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters*, 579, 5157–5162.

REPRODUCTION, PHYSIOLOGY, ANATOMY