Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
https://pjp.pakps.com

RESEARCH ARTICLE

In vitro Antibacterial Activity of Tropical Herbal Plants Combined with Silage Juice Against Bacterial Pathogen: Initial Screening

[a]Tri R. Prihambodo*, [a]Bambang Hartoyo, [c]Nahrowi, [c]Anuraga Jayanegara, Sri Rahayu, [b]Wahyuningsih, [a]Muhamad Bata, Efka A. Rimbawanto, Ari D. Nurasih
[a] *Animal Science Faculty, Jenderal Soedirman University, Purwokerto, Indonesia.*
[b] Agricultural Development Polytechnic, Bogor, Indonesia.
[c] *Animal Science Faculty, IPB University, Bogor, Indonesia.*

Corresponding Author:
Tri Rachmanto Prihambodo, Email: tri.rachmanto@unsoed.ac.id

Article History:
Submitted: March 13, 2025; Revised: June 17, 2025; Accepted for Publication: August 25, 2025.

A B S T R A C T

This study aimed to investigate the effect of using various herbal plants in combination with fermentation treatment of silage on the inhibition of pathogenic bacteria. The research consisted of two main phases. The first phase included measuring the qualitative phytochemical profile and determining the lactic acid bacteria content of 7 different types of silage juice. The second phase involves quantitative phytochemical profiles and antioxidant capacity against pathogenic bacteria. Descriptive analysis method was used in this research. The research results showed that silage juice from *Dracaena fragrans, Tithonia diversifolia*, and *Acanthus ilicifolius* L leaves was the main choice because of its abundant lactic acid bacteria and phytochemical content. *Dracaena fragrans* silage juice, which contained flavonoids and tannins in amounts of 0.02 and 0.01 ml/100 mg, together with antioxidants of 926 ppm, is able to produce antibacterial compounds with a strong category for inhibiting pathogenic bacteria. Thus, it can be concluded that combination of plants and harvest period is believed to have the ability to inhibit bacteria well if the maximum harvest period is 20 days and the right solvent is used to stop overfermentation process. Silage juice with *Dracaena fragrans*-based is able to provide strong inhibition against pathogenic bacteria based on its phytochemical, lactic acid bacteria and antioxidant content.

Keywords: Antioxidant Capacity, Leaves, Pathogen Bacteria, Phytochemical.

INTRODUCTION

Conventional or synthetic antibiotic usage had become the main focus several years ago in terms of searching for new solutions to overcome bacterial infections that are increasingly difficult to control (Wu *et al.* 2019). In addition, the development of bacterial resistance due to conventional antibiotics has become a new challenge for farmers in Indonesia (Brabander *et al.,* 2022; Gandra *et al.,* 2020). Antibiotics are antibacterials made from yeast, which is synthesized *de novo,* and sometimes antibiotics can be used as antibacterials, analogically.

An interesting approach as a solution in the last decade has been natural antibacterial material exploration. Indonesia is known as a mega biodiversity nation, which has about 30.000 species, of which 25.000 are flowering plants, of which 55% are endemic to Indonesia (Salim 2017), so Indonesia is one of the agrobiodiversity centers of cultivar plants in the world.

The use of natural antibiotics has been reported to kill pathogenic bacteria that circulate in animal livestock, specifically poultry (Prihambodo *et al.,* 2021). Use of antibacterials such as phytochemicals (Hidayat *et al.,* 2021), lactic acid bacteria (Hartoyo *et al.,* 2023), and enzymes (Egorov *et al.,* 2018), whether used individually or in combination. The material of each material or its combination should be easy and cheap to get (Gupta and Sharma, 2022). To fulfil that requirement, a combination of silage juice and herbal plants is needed. Silage juice are by-products of silage making due to the fermentation process, which produces liquids that contain lactic acid bacteria and enzymes to kill pathogen bacteria (Prihambodo *et al.,* 2019), according to what previous researchers did.

The combination based on the available natural antibiotics is inadequate for individual use, even though much literature has been reported (Chanda and Rakholiya, 2011; Liu *et al.,* 2022). So, the aim of this research is to explore, profile, and evaluate silage juice with selected herbal plants based on phytochemical content and antioxidant activity to support antibacterial activity.

Material and Method

Plant Material: In this study, corn (*Zea mays*) and locally herbal plants corn plant (*Dracaena fragrans*), aralia (Polyscias scutellaria), mexican sunflower (*Tithonia diversifolia*), star flower (*Hippobroma longiflora*), orchid (*Bauhinia purpurea*), hibiscus (*Hibiscus tileaceus*) and sea holly (*Acanthus ilicifolius*) were collected from the area around Jenderal Soedirman University. The plants were harvested at random ages and fresh and especially for Z. mays, it was separated according to its parts and cut into about 3 cm with a harvest age of around 60 days.

Silage and Silage Pressing Process: Dry matter was utilized as an early test to determine the formula for creating silage for the plants. The whole *Zea mays* was divided into pieces, and each leaf was examined individually. The silage formula combines each herbal plant with whole corn maize at a 50:50 (w/w) ratio in DM basis. Silage making began by combining the materials according to the formula and placing them in a plastic container. Initial phytochemical analysis with 0 (not fermented) and 30 days of harvest age, followed by additional analysis with 0, 10, 20, and 30 days of age. On the day of harvest, the silage was pressed to remove the fluids produced by the fermentation process refers to silage juice

Microbial Strain: Three strains were divided into two types namely gram positive (*Bacillus cereus*) and gram negative (*Escherichia coli* and *Salmonella thypimurium*). All organisms were obtained and well prepared onto Seafast Center IPB University.

Phytochemical Screening: Quantitative and qualitative screening for phytochemical used in this investigation. Qualitative is an initial screening of phytochemical to divide seven herbal plants into three selected herbal plants. Zero (non-fermented plant) and thirty days' age of silage consisted of alkaloid, flavonoid, phenol hydroquinone, steroid, triterpenoid, tannin and saponin according to Chaudhary *et al.* (2010) was analysed. Three selected plants after qualitative screening were analysed consist of flavonoid, tannin and saponin (Khumaida *et al.,* 2019) which using 10, 20 and 30 days' age of silage.

STATISTICAL ANALYSIS

Description test was used to select three best leaves qualitatively and phytochemical. Further test, for quantitatively phytochemical, antioxidant and microbial acivitiy used analysis of variance (ANOVA) with confidence level at 95% using SAS On Demand Academic and Duncan test used as post hoc.

RESULTS AND DISCUSSIONS

Initial Phytochemical Screening: In the first phase, pre-screening of herbal plants that combined with silage juice consisted of alkaloid, flavonoid, phenol hydroquinone, steroid, triterpenoid, tannin and saponin were tested qualitatively. The examination was carried out by reacting herbal plant extracts (0 day) and silage results (30 days) by looking at the color intensity shown. The stronger the color shown, the more phytochemical content. The results can be seen in Table 1.

Based on the data above, alkaloid can be found in *Tithonia diversifolia, Dracaena fragrans, Polyscias scutellaria, Hippobroma longiflora, Bauhinia purpurea and Acanthus ilicifolius* L with silage extract mixture. Saponin was found in four herbal plants, *Polyscias scutellaria, Hippobroma longiflora, Bauhinia purpurea* and *Acanthus ilicifolius* L. with silage extract mixture. Meanwhile flavonoid, triterpenoid and tannin can be found in all herbal plant tested. Plus, icon showed greater phytochemical content, *Hibiscus tileaceus* L *and Bauhinia purpurea* had greater triterpenoid, *Dracaena fragrans, Hippobroma longiflora*, *Bauhinia purpurea* and *Acanthus ilicifolius* Linn had greater tannin while *Hippobroma longiflora* had greater saponin than other herbal plants in silage juice mixture

Nevertheless, fermentation or silage reduces phytochemical content. Water plays an important role to bind phytochemical while press process occurs. Unfortunately, water in odd moment is not the suitable solvent for all phytochemical. Tannin more suitable extracted using chloroform, flavonoid better result with methanol (Kumar et al 2023) and triterpenoid using ethyl acetate (Ernawati et al 2019) even saponin and alkaloid can be extracted using various solvent included water (Kumar et al 2023). Beside of that, there are several reasons phytochemical reduces due to fermentation.

Table 1. Qualitative phytochemical and lactic acid bacteria content of pre and post fermentation of material

Material	Age of Silage (d)	Phytochemical Content					LAB (log cfu/ml)
		Alkaloid	Flavonoid	Triterpenoid	Tannin	Saponin	
Tithonia diversifolia	0	++	++	+	+	+	-
	30	-	+	+	+	-	8.20
Hibiscus tiliaceus L	0	-	+	+++	+	-	-
	30	-	+	-	-	-	8.15
Dracaena fragrans	0	++	+	+	++	-	-
	30	-	+	+	+	-	8.24
Polyscias scutellaria	0	++	+	+	+	+++	-
	30	-	-	+	+	-	7.91
Hippobroma longiflora	0	++	+	++	++	++	-
	30	-	+	-	-	-	8.37
Bauhinia purpurea	0	+	+	+++	++	++	-
	30	-	+	-	-	-	8.33
Acanthus ilicifolius L.	0	++	+	+	++	++	-
	30	+	-	+	-	-	8.21

Note: 0 d = non-fermented; 30 d = fermented (30 days). Symbols: "–" = not detected, "+" = low, "++" = moderate, "+++" = high.

To produce about 200-ml fluid silage juice, combination of whole Zea mays and herbal plants should be made from 1-kg as feed material. Press process to produce juice solvent cannot carry all the phytochemical content of the combination. Extraction process is certain component separation with suitable solvent. So, not all phytochemicals can be transferred to water solvents. There are still many phytochemicals trapped are not bound to the solvent. Even though there are several reasons decrease of phytochemical that reported by Hur et al (2014) and Tian *et al.* (2018).

Based on Table 1, several herbs plant did not give optimal effect based on phytochemical content and lactic acid bacteria such as *Polyscias scutellaria, Hibiscus tileaceus* L, *Hippobroma longiflora* and *Bauhinia purpurea*. Only *Acanthus ilicifolius* Linn*, Tithonia diversifolia and Dracaena fragrans* were selected to future examine.

Flavonoid, Tannin and Saponin Profile and Antioxidant Capacity

Because of future examine, selected silage juice was analysed continuously with the quantitative approach and antioxidant capacity of selected silage juice be equipped and can be seen in Table 2.

Table 2. Phytochemical content and antioxidant of silage juice combine with selected herbs

Silage Juice	Period of Harvest (d)	Phytochemical			Antioxidant (ppm)
		Flavonoid (ml/100ml)	Phenol (mg/100ml)	Tannin (mg/100ml)	
Tithonia diversifolia	10	0.03	99.5^{a}	0.02	977^{a}
	20	0.02	91.7^{b}	0.01	958^{a}
	30	0.02	45.3^{e}	0.01	374^{d}
Acanthus ilicifolius L.	10	0.03	74.7^{d}	0.02	896^{ab}
	20	0.02	96.2^{a}	0.01	812^{bc}
	30	0.02	85.9^{c}	0.01	637^{c}
Dracaena fragrans	10	0.03	99.5^{a}	0.01	764^{b}
	20	0.02	91.8^{b}	0.01	926^{ab}
	30	0.02	96.7^{a}	0.01	912^{ab}

Numbers with different superscripts in the same column indicate significant differences

In this stage, different period of silage harvest checked because this treatment is important due to plant material and moisture content influence the final silage yield. Main reason of the researcher performing this method is the period of harvest can determine bacteria community of silage (Estrada *et al.* 2020). According to microorganism phase with the fermentation occurs, alteration bacterial and yeast community domination that affect to O2 availability, acidity and aerobic population affect to quality of silage (Wang et al 2019).

As a result of qualitatively phytochemical and antioxidant capacity analysis showed different result of each parameter. In flavonoid and tannin content of silage juice did not give any significant ($p>0.05$) effect due to both periods of harvest and material used, contrary result showed in phenol content and antioxidant activity with significant effect ($p<0.05$). Based on those capabilities to inhibit three species of different bacteria tested showed that in 10 days' age of fermentation, inhibitory zone for Escherichia coli is 5.19, 6.78 and 8.23 mm, 20 days is 6.81, 8.49 and 10.13 mm and 30 days were 5.56, 7.08 and 6.7. In 20 days' age of fermentation, inhibitory zone for *Salmonella thypimurium* resulted 5.58, 7.55 and 9.10, 20 days were 6.90, 9.18 and 10.59 mm and 30 days is 6.97, 6.52 and 6.66 mm. In 30 days' age of fermentation, inhibitory zone for Bacillus cereus were 7.26, 8.13 and 6.63 mm, 20 days was 6.49, 9.08 and 9.96 mm, 30 days was 4.38, 6.99 and 6.65 mm for combination silage juice with *Tithonia diversifolia, Hippobroma longiflora and Dracaena fragrans*, respectively.

Based on the collected data, we may assume that longer time fermentation in this research may give worse effect to silage juice. As stated in Table 2, most of highest phenol content and antioxidant activity can be seen in shorter period of harvest that is in 10 and 20 days. The result is similar to Zhao *et al.* (2021) that reported gradually antioxidant can gradually decrease due to increasing fermentation time. Eventhough our silage juice cannot give the best result of fermentation due to unsuitable solvent, selected plants were optimized under 20 days of harvest period.

Hubert *et al.* (2008) reported the same result, there was a rise in phytochemical content in shorter time fermentation but it dipped after it. Fermentation in longer time can lost some of its phenol. The fermentation was broken due to agents such as lactic acid bacteria and their enzim of longer time of fermentation decrease these compound that combined with fermentation weakened the cell wall too, released bound phenol and it can be used to antioxidant. Besides that, lower pH and higher temperature affect to decrease of phenol content (Wijayanti & Setiawan, 2017). As we know, higher temperature is product resulted by aerobic respiration in early stage of fermentation phase. This decline does not always indicate a decrease in the ability of silage juice to inhibit bacteria. Fermentation can produce some new metabolite which not present in the original ingredients such as flavour, bioactive, antioxidant, probiotic and another compound.

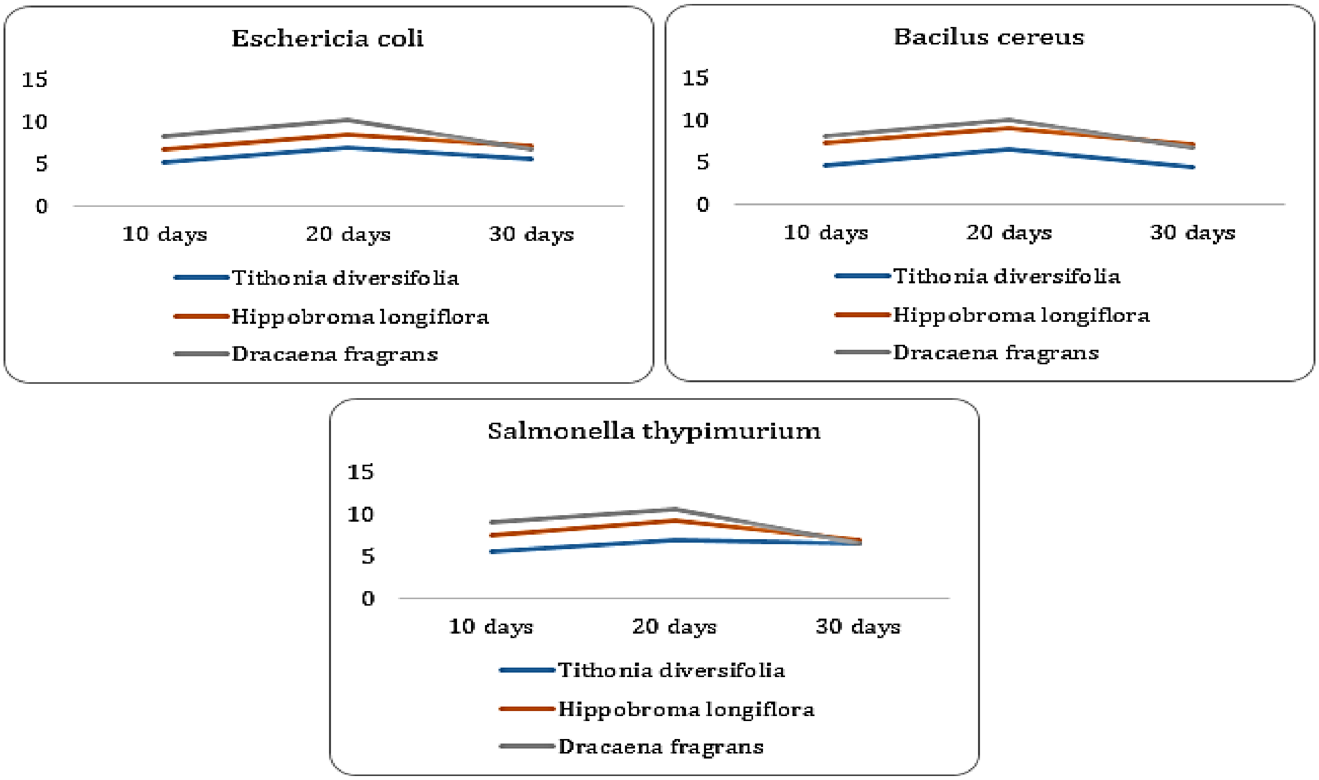

Figure 1. Pathogen bacterial inhibition of selected silage juice

Based on Figure 1, silage juice well-inhibit both bacteria gram positive and negative. *Escherichia coli, Bacillus cereus* and *Salmonella thypimurium* are pathogen bacteria which can be found in digestive tract of broiler. One of mechanism of conventional antibiotic is altering the digestive tract of pathogen bacteria which mentioned above (Mehdi *et al.,* 2018). As a follow of ANOVA result revealed that interaction of plant used and period of harvest significantly ($p<0.001$) influenced the inhibit of bacteria tested. It is because different plants and harvest times influence the microbes that take part in fermentation, the chemistry constituents of plant, and how the whole fermentation process unfolds. It is in line with the capability to inhibit tested bacteria decrease in longer period.

In 10 days' age of fermentation, inhibitory zone for Escherichia coli were 5.19, 6.78 and 8.23 mm, 20 days were 6.81, 8.49 and 10.13 mm and 30 days were 5.56, 7.08 and 6.7. In 20 days' age of fermentation, inhibitory zone for *Salmonella thypimurium* is 5.58, 7.55 and 9.10, 20 days is 6.90, 9.18 and 10.59 mm and 30 days were 6.97, 6.52 and 6.66 mm. In 30 days' age of fermentation, inhibitory zone for Bacillus cereus were 7.26, 8.13 and 6.63 mm, 20 days is 6.49, 9.08 and 9.96 mm, 30 days were 4.38, 6.99 and 6.65 mm for combination silage juice with *Tithonia diversifolia*, *Hippobroma longiflora* and *Dracaena fragrans*, respectively.

Morales et al (2003) categorize capability of compound to inhibit bacteria. Inhibition <5 mm is weak, 5-10 mm is moderate, 10-20 mm is strong and >20 mm is very strong. Based on Morales reported, in shorter time of fermentation gave better inhibition ($p<0.05$) than longer time. Best inhibition in number is in 20 days of fermentation time.

Content of tannin decreases however tannin parsed into small benefit molecules such as catechin and gallo-catechin (Shang et al 2019). Catechin is secondary metabolite produce by plants with the antioxidant capacity mean while gallocatechin is an agent with the responsibility for biological activity such as antibacterial activity (Bartosikova and Necas, 2018). Meanwhile fermentation for flavonoid can increase antioxidant activity. Mechanism of fermentation can increase antioxidant capacity reported by Hur *et al*. (2014) by generating phenolic compounds and flavonoids, releasing or synthesizing various antioxidants, and producing metal-chelating enzymes, enhancing their antioxidant properties. Chu and Chen (2006) reported the same result with our experiment. Antioxidant increases due to fermentation but tend to decrease after 15 days of fermentation.

Combination of lactic acid bacteria, phytochemical in plants and antioxidant capacity complement simultaneously to inhibit growth of harmful bacteria. Even though, silage juice process that selected in this experiment is not suitable, but silage juice still potential to use as alternative antibiotic. Phytochemical of plants has their own way to inhibit harmful bacteria. Aulifa *et al.* (2017) summarizes it when phytochemical used as antibacterial lactic acid has the ability to diffuse through microbial membranes, that in turn reduces the flexibility of bacterial cell membranes and causes an imbalance in cell fluids, leading to bacterial death. Furthermore, lactic acid can produce organic acid such as propionic acid, valerate and iso-butyrate which can decrease intracellular pH in bacteria and caused bacteria death (Ramli & Setiyono, 2015). Their combination is also complement with antioxidant of silage juice. Decrease pathogen burden and as antimicrobial agents are mostly role of antioxidant as antibacterial (Paiva and Claudia 2014). The mechanism by which antioxidants are understood as antibacterials is antioxidants have the capacity to react directly with reactive oxygen species (ROS) and deactivate them, leading to a reduction in oxidative harm to host cells and a restriction in the availability of ROS for the survival and replication of pathogens (Paiva and Claudia 2014). ROS has correlation with immunity against infection which antioxidant indirectly modulation by enhancing the host defence by influencing cytokines production.

Combination of lactic acid bacteria, phytochemical content and antioxidant in each silage juice which used wild plants reported in this examination leads researcher to more explore about plant. This result consisted of selected plants and its combination to produce silage juice reveal their potential. In this examination, *Dracaena fragrans* has capability than other two. Based on phytochemical such as tannin, flavonoid dan phenol, all of three have not big different but antioxidant become a differentiator.

CONCLUSION

Combination of plants and harvest period is believed to have the ability to inhibit bacteria well if the maximum harvest period is 20 days and the right solvent is used to stop overfermentation process. Silage juice with *Dracaena fragrans*-based is able to provide strong inhibition against pathogenic bacteria based on its phytochemical, lactic acid bacteria and antioxidant content.

REFERENCE

Aulifa D. L., S. N. Fitriansyah, S. A. Ardiansyah, D. P. Wibowo, Y. A. Julata, and D. S. Christy. 2017. Phytochemical Screening, Antibacterial Activity, and Mode of Action on *Morus nigra*. Pharmacognosy Journal, Volume *10* Article 1, 167–171. https://doi.org/10.5530/pj.2018.1.28.

Bartosikova, L., and J. Necas. 2018. Epigallocatechin gallate: a review. Veterinary Medicin. Volume 63 Article 10. 443-467. https://doi.org/10.17221/31/2018-vetmed.

Brabander J. D., Nelwan, E. J, Limato, R, Tjoa, M. M. E, Mauleti, I. Y, Mayasari, M, Firmansyah, I, Jayati, T. M, van Vugt, M, van Doorn, H. R, and Hamers, R. L. 2022. Bacterial culture use, etiology and antibiotic susceptibility of common bacterial infections in Indonesian hospital in 2019. *medRxiv*. https://doi.org/10.1101/2022.03.09.22272145.

Chanda S., and K. D. Rakholiya. 2011. Combination therapy: synergism between natural plant extracts and antibiotics against infectious disease. Science Against Microbial Pathogens.

Chaudhary S, A. Negi, and V. Dahiya. 2010. The study of in vitro antimicrobial activity and phytochemical analysis of some medicinal plants in Chamoli Garhwal Region. Pharmacognosy Journal, Volume 2 Article 12, 481–485. https://doi.org/10.1016/s0975-3575(10) 80035-5.

Chu S., and C. Chen. 2006. Effect of origins and fermentation time on the antioxidant activities of kombucha. Food Chemistry. Volume 98 Article 3. 502-507. https://doi.org/10.1016/j.foodchem.2005.05.080.

Egorov, A. M., M. M. Ulyashova, and M. Y. Rubtsova. 2018. Bacterial enzyme and antibiotic resistance. Acta Naturae. Volume 10 Article 4, 33-48.

Ernawati, E. Suprayitno, Hardoko and U. Yanuhar. 2019. Extraction of bioactive compounds fruit from Rhizophora mucronata using sonication method. IOP Conference Series: Earth and Environmental Science, Volume *236* Article 1. https://doi.org/ 10.1088/ 1755-1315/236/1/012122.

Estrada, P. A. C., J, Fernandes, E. B. Silva, P. Tizioto, S. F. Paziani, A. P. Duarte, L. L. Coutinho, M. C. Q. Verdi, and L. G. Nussio. 2020. Effect of hybrid, kernel maturity, and storage period on the bacterial community in high-moisture and rehydrated corn grain silage. Systematic and Applied Microbiology. Volume 43 and Article 5. https://doi.org/10.1016/j.syapm.2020.126131.

Gandra S., G. Alvarez-Uria, P. Turner, J. Joshi, D. Limmathurotsakul, and H. R. van Doorn. 2020. Antimicrobial resistance surveillance in low-and middle income countries: progress and challenges in eight south Asian and southeast Asian countries. Clinical Microbiology Review, Volume 33 Article 3.

https://doi.org/10.1128/cmr.00048-19.

Gupta R, and S. Sharma. 2022. Role of alternatives to antibiotics in mitigating the antimicrobial resistance crisis. Indian J. Medical. Research. Volume 156 Article 3, 464-477. https://doi.org/10.4103/ijmr.IJMR_3514_20.

Hartoyo, B., T. R. Prihambodo, Wahyuningsih, S. Rahayu, F. M. Suhartati, M. Bata, E. A. Rimbawanto. 2023. Lactobacillus as growth promoter: A meta-analysis of performance, histology, and microbiota on broiler tract digestive. Advances in Animal and Veterinary Science, Volume 11 Article 6.

Hidayat, C., A. Irawan, A. Jayangara, M. M. Sholikin, T. R. Prihambodo, Y. R. Yanza, E. Wina, Sadarman, R. Krisnan, Isbandi. 2021. Effect of dietary tannins on the performance, lymphoid organ weight, and amino acid ileal digestibility of broiler chicken: A meta-analysis. Veterinary World, Volume 14 Article 6. https://doi.org/10.14202/ vetworld.2021.1405-1411.

Hubert, J., M. Berger, F. Nepveu, F. Paul, and J. Dayde. 2008. Effect of fermentation on the phytochemical composition and antioxidant properties of soy germ. Food Chemistry. Volume 109 Article 4. 709-721. https://doi.org/10.1016/j.foodchem.2007.12.081.

Hur, S. J., S. Y. Lee, Y. C. Kim, I. Choi, and G. B. Kim. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. Food Chemistry, Volume 160, 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112

Khumaida, N, M. Syukur, M. Bintang, and W. Nurcholis. 2019. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of Curcuma aeruginosa accessions growing in west java, Indonesia. Biodiversitas, Volume 20 Article 3, 656–663. https://doi.org/10.13057/biodiv/d200306

Kumar, A, P. Nirmal, M. Kumar, A. Jose, V. Tomer, E. Oz, C. Proestos, M. Zeng, T. Elobeid, V. Sneha and F. Oz. 2023. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. In *Molecules,* Volume 28 Article 2. MDPI. https://doi.org/10.3390/molecules28020887.

Liu, Y., W. Wang, M. Guo, Z. Xu, Y. Yang, L. Yu, Y. Li, L. Hu, Y. Ye, and J. Li. 2022. The analysis of drug-resistant bacteria from different regions of anhui in 2021. *Infect Drug Resist.* Volume 15 Article 15, 7537-7553. https://doi.org/10.2147/IDR.S393760.

Mehdi, Y., M. Letourneau-Montminy, M. Gaucher, Y. Chorfi, G, Suresh, T, Rouissi, S. K. Brar, C. Cote, A. A. Ramirez, and S. Godbout. 2018. Use of antibiotics in broiler production: global impacts and alternatives. *Anim. Nutr.* Volume 4 Article 2, 170-178. https://doi.org/10.1016/j.aninu.2018.03.002.

Morales G, A. Paredes, L. A. Loyola and J. Borquez. 2003. Secondary metabolites from four medicinal plants from northern Chile: Antimicrobial activity and biotoxicity against Artemia salina. Journal of the Chilean Chemical Society, June 2014. https://doi.org/10.4067/S0717-97072003000200002.

Paiva, C. N., and M. T. Bozza. 2014. Are reactive oxygen species always detrimental to pathogens? *Antioxid Redox Signal*. Volume 20 Article 6. 1000-1037. https://doi.org/10.1089/ars.2013.5447.

Prihambodo, T. R., M. M. Sholikin, N. Qomariyah, A. Jayanegara, I. Batubara, D. B. Utomo, Nahrowi. 2021. Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. Animal Bioscience, Volume 34 Article 3. https://doi.org/10.5713/ajas.20.0379

Prihambodo, T. R, Nahrowi, A. Jayanegara. 2019. Antibacterial activity and phytochemical content of silage juice from tropical herbal leaves. IOP Conference Series: Material Science and Engineering. Volume 546 Article 4. https://doi.org/10.1088/1757-899X/546/4/042032.

Ramli, N., and A. Setiyono. 2015. *Karasteristik jus dari silase jagung berbeda umur serta kemampuannya dalam menghambat e. Coli dan salmonella* sp (Juice characteristics of corn silage from different age and its cap. February.

Salim Z. 2017. Info komoditi I.

Shang, Y. F., H. Cao, Y. L. Ma, C. Zhang, F. Ma, C. X. Wang, X. L. Ni, W. J. Lee and Z. J. Wei. 2019. Effect of lactic acid bacteria fermentation on tannins removal in Xuan Mugua fruits. Food Chemistry, Volume 274 118–122. https://doi.org/10.1016/j.foodchem.2018.08.120

Svensson L, B. Sekwati-Monang, D. L. Lutz, R. Schieber, and M. G, Gänzle. 2010. Phenolic acids and flavonoids in nonfermented and fermented red sorghum (Sorghum bicolor (L.) Moench). Journal of Agricultural and Food Chemistry, Volume 58 Article 16, 9214–9220. https://doi.org/10.1021/jf101504v

Tian J, R. Na, Z. Yu, Z. Liu, and Y. Yu. 2018. Inoculant effects on the fermentation quality, chemical composition and saponin content of lucerne silage in a mixture with wheat bran or corn husk. Animal Production Science, Volume 58 Article 12, 2249. https://doi.org/10.1071/an16407.

Wang W., Z. Tan, L. Gu, H. Ma, Z. Wang, L. Wang, G. Wu, G. Qin, Y. Wang, and H. Pang. 2022. Variation of microbial community and fermentation quality in corn silage treated with lactic acid bacteria and *Artmisia argyi* during aerobic exposure. *Toxins*. Volume 14 Article 5. https://doi.org/10.3390/toxins14050349.

Wijayanti E, and N. Setiawan. 2017. The effect of lactic acid fermentation on fig (*Ficus carica*) fruit flavonoid. *Journal of Biological Research*, *23*(1), 39–44.

Wu, S, A. Li, X. Zhao, C. Zhang, B. Yu, N. Zhao, and F. Jian-Xu. 2019. Silica-coated gold-silver nanocages as photothermal antibacterial agents for combined anti-infective therapy. ACS Applied Materials & Interfaces. https://doi.org/10.1021/acsami.9b01149

Zhao, Y., A. S. Eweys, J. Zhang, Y. Zhu, J. Bai, O. M. Darwesh, H. Zhang, and X. Xiao. 2004. Fermentation affects the antioxidant activity of plant-based food material through the release and production of bioactive components. Antioxidants. Volume 10 Article 12. https://doi.org/10.3390/antiox10122004.

Contribution of Authors:

Tri R. Prihambodo	:	Conceptualization of the research, Writing – Original Draft, Data analyst
Bambang Hartoyo	:	Supervision
Nahrowi	:	Conceptualization of the research
Anuraga Jayanegara	:	Conceptualization of the research, Methodology
Sri Rahayu	:	Supervision
Wahyuningsih	:	Writing – Review and editing
Muhamad Bata	:	Supervision
Efka A. Rimbawanto	:	Supervision
Ari D. Nurasih	:	Writing – Original draft