Original Article

Productivity of Young Broilers Fed *Phyllanthus niruri* Linn and *Melaleuca cajuput* Leaf Meal as a Phytogenic Feed Additive

Listya Purnamasari[1,4] Melinda Erdya Krismaputri[1] Yusup Sopian[2]

Joseph F. dela Cruz[3] Seong Gu Hwang[4*]

Abstract

Feed additives from medicinal plants can be used as alternative antibiotics to optimize productivity, growth and health in livestock. Medicinal plants *Phyllanthus niruri* Linn (Meniran) and *Melaleuca cajuput* (Kajuput) as alternative growth promoters contain antioxidants and antimicrobials to improve the quality of animal products. The aim of this study was to evaluate the effect of medicinal plant leaf meals as phytogenic feed additives on broilers' productivity and internal organ parameters. For 21 days feeding, 80 1-day-old Loughmann broilers (MB202) unisex were distributed in 4 treatments with 4 pens per treatment and 5 birds per pen. The dietary treatments were: T1 (antibiotics), T2 (2% Meniran), T3 (2% Kajuput) and T4 (1% Meniran + 1 % Kajuput). There were no significant differences (P<0.05) in body weight gain, feed intake, feed conversion ratio, average weights of the carcass and internal organ weight by feed additive supplementation. However, the bursa fabricius weight increased significantly (P<0.05) with the feed additive. In conclusion, medicinal plant leaf meal supplementation compared to commercial antibiotics has the same effect on growth performance, carcass and internal organ weight. The combination of meniran and kajuput leaf meal as phytogenic feed additives increases the weight of the bursa fabricius which indicates improved health status.

Keywords: growth performance, carcass, phytogenic, organ weight, meniran, kajuput

[1]*Animal Husbandry Study Program, Faculty of Agriculture, University of Jember, Jember, 68121, Indonesia*
[2]*Animal Husbandry Study Program, Faculty of Agriculture Muhammadiyah Education University of Sorong, West Papua, 98418, Indonesia*
[3]*Department of Basic Veterinary Science, College of Veterinary Medicine, University of the Philippines Los Baños, College, Laguna 4031, Philippines*
[4]*School of Animal Life Convergent Science, Hankyong National University, Anseong 17579, Republic of Korea*
***Correspondence**: sghwang@hknu.ac.kr* (S.G. Hwang)
Received January 4, 2023
Accepted May 14, 2023
https://doi.org/10.14456/tjvm.2023.13

Introduction

Efficient livestock feeding can increase productivity and reduce production costs to increase profits. Feed additives can improve livestock nutritional health, productivity and fulfilment (Mehdi *et al* 2018). Antibiotics are one of the feed additives that are widely used throughout the world. However, the use of antibiotics in developed countries has been abandoned as a feed additive since 2006 because it is feared that it will cause antibiotic residues in livestock products, causing allergies to consumers (Diarra *et al*, 2010), disrupt the balance of microorganisms in the digestive tract and the resistance of microorganisms to antibiotics (Ronquillo dan Hernandez, 2017).

Medicinal plants that have long been known to be efficacious for preventing disease in humans are also believed to be used as multi-function phytobiotics (MFP) natural feed additives (phytogenic feed additives). The use of phytogenic feed additives in animal feed can replace the use of antibiotics (Olawuwo *et al*, 2022). It can prevent the pathogenic bacteria growth in the feed so that the number of non-pathogenic bacteria in the feed is higher (Abd El-Hack *et al*. 2022). In addition, it can also increase livestock productivity as a growth promoter, increase feed digestibility, detoxify toxins, increase immunity, reduce morbidity and mortality and prevent and treat livestock diseases (Namdeo *et al*, 2020).

Some medicinal plants have functioned as antioxidant, antifungal, anti-inflammatory, antiviral, antimicrobial, and immunostimulatory properties (Krishan &Narang, 2014) such as *Phyllanthus niruri* Linn (meniran) (Fang *et al* 2008) and *Melaleuca cajuput* (kajuput) (El-Hack *et al*. 2022; Talazadeh and Mayahi, 2017). The secondary metabolites of medicinal plants are the form of active ingredients or bioactive components that have an important role in livestock survival. The compounds of phytogenic are considered to be easy to use, safe and environmentally friendly (Akdemir *et al*., 2012). One plant usually has more than one pharmacological effect due to producing more than one type of secondary metabolite (organic acids, phytoalexins, essential oils, etc.) (Brewer, 2011). Several types of active ingredients from the combination of medicinal plants show more effectiveness than the use of a single active ingredient (Günther dan Ulfah 2003).

Considering this, research is needed on the use of phytogenic feed additives from medicinal plants that contain phytonutrients, phytochemicals and essential oils. The aim of the study was to evaluate the effect of medicinal plant leaf meal (*Phyllanthus niruri* Linn and *Melaleuca cajuput*) as phytogenic feed additives on broilers' productivity and internal organ parameters.

Materials and Methods

For 21 days feeding, 80 1-day-old Loughmann broiler chickens (MB202) unisex were distributed in 16 pens with 5 birds per pen. Broilers were assigned to 4 treatments: T1 (diet with antibiotics (tetracyclines 100 ppm), T2 (diet with 2% Meniran), T3 (diet with 2% Kajuput) and T4 (diet with 1% Meniran + 1 % Kajuput). The diets and water were offered ad libitum. The results of the antioxidant test (polyphenols) of meniran and kajuput leaf meal are shown in Table 1. and the component bioactive are shown in Table 2. The broilers were fed for 21 days (01-21 days of age). The feed nutrients and composition of the basal diet is shown in Table 3.

Table 1 The results of the antioxidant test (polyphenols)

Item	Polyphenol (%)
Phyllanthus niruri Linn leaf meal (Meniran)	12.53
Melaleuca cajuput leaf meal (Kajuput)	8.62

Table 2 Component bioactive of Phyllanthus niruri and Melaleuca cajuput

Component bioactive	Component bioactive	Function	source
Phyllanthus niruri	Lignans: phyllanthin and hypophyllanthin	Anti-viral and anti-inflammatory	Sibiya *et al*., 2020; Sagar *et al*., 2004
	Flavonoids: Rutin, Niruriflavone and Quercetin	Anti-oxidant, anti-fungal and anti-inflamatory	Bagalkotkar *et al*., 2006; Than et al 2006
	Alkaloids: Norsecurinine	Anti-bacterial	Bagalkotkar *et al*., 2006
	Tannin: Corilagin	Anti-fungal	Zhao *et al*., 2016
	Terpenes: p-Cymene	Anti-oxidant and anti-microbial	Medeiros *et al*., 2003
Melaleuca cajuput	Terpenoids: cineol, limonene, 4-terpineol, α-terpineol, α-pinene	Anti-bacterial and anti-microbial	Ukit *et al*., 2019; Widiana *et al*., 2015;
	Linalool	Anti-inflammatory, anti-oxidant and spamolytic	Isah *et al*., 2023
	caryphyllene and caryophyllene oxide	anti-inflammatory, anti-fungal	Peana & Mario, 2008 Munoz *et al*., 2012

Table 3 Feed composition and nutrient content of the basal experimental diet

Ingredients	***Percentage (%)***
Maize	56,70
Soybean meal	34,22
Palm olein	2,90
Dicalcium phosphate	1,35
Choline	0,005
DL-methionine	0,28
Premix*	0,51
L-lysin HCl	0,20
L-threonine	0,07
Bone meal	2,00
Phytogenic feed additives	2,00
Analyzed nutrient content	
ME (kcal/kg)	3014,81
Crude Protein (%)	21,77
Lys (%)	1,27
Met (%)	0,59
Trp (%)	0,22
Ca (%)	0,95
P total (%)	0,76
K (%)	0,87
Cl (%)	0,30
Na (%)	0,19
Zn (ppm)	85,61

Performance parameters: Productive performance that is measured weekly is final body weight, daily weight gain, feed intake and feed conversion ratio. Mortalities are recorded at the occurrence. On arrival the day-old-chick (DOC) was weighed as initial body weight and during the 3-week trial at the end of each week, the body weight gain was recorded. The difference in weight gain in that week and the previous week is the weight gain.

$$\text{Daily body weight gain (g)} = \frac{final\ body\ weigt\ (g) - Initial\ weight\ gain\ (g)}{total\ number\ of\ the\ experiment\ (21\ days)}$$

Feed intake was measured from the differences between the given feed and the leftover every week. The ratio of their feed intake to weight gain was calculated to get the feed conversion ratio (FCR) of the chickens as the formula below

$$\text{FCR} = \frac{Total\ feed\ intake\ (g)}{total\ body\ weight\ gain\ (g)}$$

Carcass weight and internal organs weight: At the end of the experiment (21 d of age), 4 chickens per treatment group (1 chicken/pen) were randomly selected and slaughtered. The weight of their carcasses and internal organ weight (proventriculus, ventriculus, heart, liver, bursa fabricius, spleen, duodenum, jejunum, ileum, colon) were weighed using a sensitive weighing scale (0.01g). After evisceration, the carcasses were weighed and recorded as relative weights (% live weight).

Statistical analysis: Data performance parameters and internal organ weights data were analyzed by one-way ANOVA (P<0.05) followed by Duncan Multiple Range Test (DMRT) using statistical package SAS 9.4.

Results

The chickens in this research were healthy and there were no dead chickens during the entire experimental period of 21 days feeding. The growth performance data of the broiler chickens is shown in Table 4. The supplementation of the phytogenic feed additives had no significant effect (P>0.05) on body weight, daily weight gain, feed intake and feed conversion ratio. The results for the internal organ weights of the chickens are shown in Table 5. The supplementation of phytogenic feed additive had no significant effect on the carcasses and the internal organ weights of chicken (proventriculus, ventriculus, heart, liver, bursa fabricius, spleen, duodenum, jejunum, ileum, colon). However, the bursa fabricius weight was increased significantly (P<0.05) by the feed additive.

Table 4 Effect of different phytogenic feed additives on growth performance of broiler chickens

Parameters	**Treatments[1]**				***P* value**
	T1	**T2**	**T3**	**T4**	
Initial BWT (g)	44.45±1.29	43.80±1.50	43.20±2.06	42.40±2.38	0.644
FI (g)	213.75±24.80	239.50±24.90	226.50±43.65	215.75±35.90	0.755
DWG (g)	82.15±17.41	98.79±15.21	91.44±33.33	87.46±15.02	0.731
FCR	2.64±0.33	2.44±0.25	2.69±0.81	2.48±0.31	0.791
Final BWT (g)	519.50±14.84	506.25±4.78	509.00±9.12	512.2.00±10.23	0.203

[1]T1: diet + tetracycline 10mg/kg (control), T2: diet + 2% Meniran, T3: diet + Kajuput, T4: diet + 1% Meniran + 1% Kajuput

Table 5 Effect of different phytobiotics on carcass and internal organs weight of broiler chickens

Parameters	Treatments[1]				*P* value
	T1	T2	T3	T4	
Carcass (%)	62.7±2.9	64.2±1.0	63.9±1.1	63.6±0.9	0.218
Liver (g)	15.86±0.82	15.91±3.06	18.08±6.01	13.10±2.25	0.175
Heart (g)	3.65±0.57	4.00±0.76	3.82±0.43	3.45±0.56	0.653
Bursa (g)	1.26±0.05a	0.67±0.17b	0.83±0.27b	0.91±0.17ab	0.000
Spleen (g)	0.71±0.12	0.57±0.16	0.90±0.66	0.48±0.17	0.631
Duodenum (g)	4.37±0.48	3.53±0.92	3.51±0.41	3.16±0.46	0.080
Jejunum (g)	11.69±1.43	11.05±1.44	11.03±1.47	10.16±3.14	0.569
Ileum (g)	11.52±3.79	10.45±3.79	11.16±0.66	7.78±1.19	0.177
Colon (g)	1.18±0.15	1.05±0.15	1.65±0.50	1.58±1.44	0.539
Proventriculus	4.35±0.96	4.24±0.65	3.83±0.25	2.99±0.56	0.167
Ventriculus (g)	16.63±2.06	16.72±2.46	17.77±4.36	14.49±1.49	0.544

[1]T1: [1]T1: diet + tetracycline 10mg/kg (control), T2: diet + 2% Meniran, T3: diet + Kajuput, T4: diet + 1% Meniran + 1% Kajuput
Numbers followed by different letters show a significant difference according to the DMRT range test ($P<0.05$)

Discussion

Natural compounds from plants are often used in the production of some pharmaceutical/cosmetic products and the agricultural industry. One of the *Melaleuca* species, *Melaleuca cajuput* (Kajuput) is commonly found in Indonesia. A number of investigations have revealed that the *Melaleuca cajuput* oil from leaves has antibacterial, antioxidant, antifungal, relieving effects (Pujiarti *et al*., 2012) and monoterpenes are the main component in the oil (Siddique *et al*., 2020).

The inclusion of phytogenic feed additives (PFA) in this study had the same result as Hafeez *et al*., (2016) that broiler chicken feed containing essential oils in a powdered form characterized by anethole and menthol did not affect the growth performance. Supporting other research by Fornós *et al*., (2022) on young broiler chickens fed with PFA (cinnamon, oregano, citrus peel) it did not influence the average daily gain but improved the feed conversion ratio. However, different results show PFA including cinnamaldehyde, carvacrol and capsicum oleoresin increases the nutritional value of diets and improves growth performance due to the anti-inflammatory effect (Pirgozliev *et al*. 2019). Another study shows that the addition of commercially available PFA (Digestarom®, Austria) on turkeys had a positive effect on body weight gain on day 14 and day 48 but no effect on day 28 (Zumbaugh *et al*, 2020).

This research was conducted for 21 days so the suggestion is to provide phytogenic feed additives until the end of the period. This research had no significant effect on body weight, daily weight gain, feed intake and FCR for all treatments of PFA and the same as a positive control with a commercial antibiotic. The PFA from meniran and cajuput leaf meal can substitute for commercial antibiotics. The effect of PFA in maintaining the same body weight as the antibiotic control group by improving feed efficiency mediated through feeding is related to hypothalamic neuropeptide modulation (Flees *et al*., 2020).

Treatment 4 had a bigger weight of bursa fabriscius than treatment 3 and treatment 2 but treatment 1 had the biggest. Treatment 4 was no different from Treatment 1. The bigger the bursa fabriscius, the higher the body's immune system. The immune system in poultry is closely related to the function of several lymphoid organs, one of which is the bursa fabrisius. The bursa fabrisius serves as a place for the maturation of cells from the antibody-forming system in chickens that can destroy antigens that enter the body (Purnamasari *et al*., 2020). Weight increase in the bursa fabricius may be a proof of the maturation of a greater number of T lymphocytes (CD4 and CD8) and lymphocyte B (indirectly through antibody production), suggesting enhanced development of adaptive immunity. However, an increase in the weight of this organ does not necessarily reflect a greater number of lymphoid cells (Guimarães *et al*., 2014). The anatomical and physiological development of the bursa can be influenced by the environment such as stress, poor hygiene, vaccination and pathological conditions due to disease. The lymphoid organs weight such as the bursa fabricius can be measured and reflects the body's ability to produce lymphoid cells in an immune response (Tabeekh dan Mayah 2009). The bursa fabricius produces B-lymphocytes and distributes them to the germinal center in lymphoid storage. This organ is the primary lymphoid organ that produces immunoglobulins in young chickens (Wardani 2009). The bioactive compound of the *Phyllanthus niruri* Linn and *Melaleuca cajuput* stimulates the activity of lymphocyte proliferation and maturation (Jantan *et al*, 2019; Qu *et al*, 2019).

The activity of phytogenic plants depends on bioactive compounds that can provide antimicrobial properties in the intestines of animals (Olayemi *et al*., 2020). *Phyllanthus niruri* Linn extract contains, tannin, niranti, filokrisna, hypophilantin, pseudokhiratin and nirurin flavonoids and philanthine which have been mass-produced for immunomodulatory drugs (Suhirman dan Winarti 2010) and enhance antibody response (Koutsos dan Klasing 2008). *Phyllanthus niruri* has antibacterial activity against a variety of pathogenic bacterial or fungal strains and the best positive effect is obtained from the nanoemulsion method (Pathania *et al*. 2022). *Melaleuca* has antifungal activity and has been developed in the prevention of candida infections (Zhang *et al*, 2018). Our results are the same as the findings of Amad *et al*. (2011), that showed that the supplementation PFA containing thyme and star anise essential oils as lead active components, did not significantly affect the liver, heart, spleen and kidneys. The internal organ weight was normal weight.

Bioactive compounds feed supplementation improves the derived product quality and some bioactive compounds are easily degraded and confer an unpleasant taste on feed (Tolve *et al*, 2021). Phytochemical components of herbal medications when given in traditional dosage forms have stability, low bioavailability and distribution. As a result, more research is needed to cover the way into the formulation of different plant extract methods or supplementation as feed additives.

In conclusion, the supplementation of *Phyllanthus niruni* Linn and *Melaleuca cajuput* leaf meal as a phytogenic feed additive did not improve the body weight gain, feed intake, FCR, carcass and of internal organ weight. However, phytogenic feed additive provides the same performance as the use of commercial antibiotics and improved health by increasing the weight of the bursa fabricius with the combination of phytogenic feed additives. This research was conducted during the starter period (21 days) so the suggestion is to provide phytogenic feed additives until the finisher period.

Acknowledgements

The study was financed by the Institute for Research and Community Service to the Community (LP2M), University of Jember, through Grant Number SP DIPA: 042.01.2.400922/2018.

References

Abd.El-Hack ME, El-Saadony MT, Saad AM, Salem HM, Ashry NM, Abd.Ghanima MMA, Shukry M, Swelum AA, Taha AE, El-Tahan AM, AbuQamar SF & El-Tarabily KA (2022). Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poultry Science 101(2): 1-21 https://doi.org/10.1016 / j.psj.2021.101584

Akdemir F, Orhan C, Sahin N, Sahin Dr K, Hayirli A (2012). Tomato powder in laying hen diets: effects on concentrations of yolk carotenoids and lipid peroxidation. British Poultry Science 53(5): 675-680 https://doi.org/10.1080/00071668.2012.729142

Amad AA, Manner K, Wendler KR, Neumann K & Zentek J (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poultry Science 90(12): 2811-2816. https://doi.org/10.3382/ps.2011-01515

Bagalkotkar G, Sagineedu SR, Saad MS & Stanslas J (2006). Phytochemicals from Phyllanthus niruri Linn. and their pharmacological properties: a review. Journal of Pharmacy and Pharmacology, 58: 1559–1570

Brewer MS (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications Comprehensive Review in Food Science and Food Safety, 10:221-247 https://doi.org/10.1111/j.1541-4337.2011.00156.x

Fang S, Rao YK & Tzeng Y (2008). Antioxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from Phyllanthus urinaria. Journal of Ethnopharmacology 116(2): 333-340 https://doi.org/10.1016/j.jep.2007.11.040

Flees J, Greene E, Ganguly B, Dridi S (2020). Phytogenic feed- and water-additives improve feed efficiency in broilers via modulation of (an)orexigenic hypothalamic neuropeptide expression. Neuropeptides, 81:1-11 https://doi.org/10.1016/j.npep.2020.102005

Fornós M, Verdú M, Soldevila X, Farré G, Salesa R, Rafaela J (2022). The effect of a phytogenic feed additive on performance and nutrient retention of young broiler chickens. Animal - science proceedings, 13(3): 431-432. https://doi.org/10.1016/j.anscip.2022.07.162

Guimarães JB, dos Santos EC, Dias ES, Bertechini AG, da Silva Avila CL & Dias FS (2014). Performance and meat quality of broiler chickens that are feddiets supplemented with Agaricus brasiliensis mushrooms. Tropical Animal Health and Production 46(8). http://dx.doi.org/10.1007/s11250-014-0655-9

Hafez A, Manner K, Schieder C & Zantek J (2016). Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. Poultry Science 95 (3): 622-629. https://doi.org/10.3382/ps/pev368

Isah M, Rosdi RA, Wahab WNAWA, Abdullah H, Sul'ain MD & Ishak WRW (2023). Phytoconstituents and biological activities of Melaleuca cajuputi Powell: A scoping review. Journal of Applied Pharmaceutical Science. 13(1): 010-023 https://doi.org/10.7324/JAPS.2023.130102

Jantan I, Haque MA, Ilangkovan M & Arshad L (2019). An insight into the modulatory effects and mechanisms of action of Phyllanthus species and their bioactive metabolites on the immune system. Frontiers Pharmacology 10. https://doi.org/10.3389/fphar.2019.00878

Koutsos EA & Klasing KC (2008). Avian Immunology. Boston: Elsevier Publishing.

Krishan G. & Narang A (2014). Use of essential oils in poultry nutrition: A new approach. Journal of Advance Veterinary and Animal Research. 1: 156-62. https://doi.org/10.5455/javar.2014.a36

Medeiros JR, Campos LB, Mendonca SC, Davin NB & Lewis NG (2003). Composition and antimicrobial activity of the essential oils from invasive species of the Azores, Hedychium gardnerianum and Pittosporum undulatum. Phytochemistry 64: 561–565 https://doi.org/10.1016/s0031-9422(03)00338-8

Mehdi Y, Letourneau-Montminy M, Gaucher M, Chorfi Y, Suresh G, Rouissi T, Brar SK, Cote C, Ramirez AA & Godbout S (2018). Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition 4(2): 170-178 https://doi.org/10.1016/j.aninu.2018.03.002

Munoz BAS, Maria IA, Beatriz KD, Jose FR & Blas LH (2012). The Sesquiterpenes β-Caryophyllene and Caryophyllene Oxide Isolated from Senecio salignus Act as Phytogrowth and Photosynthesis Inhibitors Molecules 17(2): 1437-1447 https://doi.org/10.3390/molecules17021437

Namdeo S, Baghel R, Nayak S, Khar A, Pal RP, Chaurasiya A, Thakur S & Reddy B (2020).

Essential oils: a potential substitute to antibiotics growth promoter in broiler diet. Journal Entomology and Zoology Studies. 8 (4),1643-1649

Olawuwo OS, Abdalla MA, Muhling KH & McGaw LJ (2022). Proximate analysis of nutrients and in vitro radical scavenging efficacy in selected medicinal plant powders with potential for use as poultry feed additives. South African Journal of Botany 146: 103-110 https://doi.org/10.1016/j.sajb.2021.09.038

Olayemi WA, Williams GA, Olatidoye OP & Omofunmilola EO (2020). Influence of dietary inclusion of phytobiotics on growth performance, carcass, and organ weight of broiler chickens. Journal of Agriculture and Food Sciences 18(2): 26-38.

Pathania R, Najda A, Chawla P, Kausik R & Khan MA (2022). Low-energy assisted sodium alginate stabilized Phyllanthus niruri extract nanoemulsion: Characterization, in vitro antioxidant, and antimicrobial application. Biotechnology Reports, 33 https://doi.org/10.1016/j.btre.2022.e00711

Peana AT & Mario DLM (2008). Linalool in Essensial Plant Oils: Pharmacological Effects. Botanical Medicine in Clinical Practise 79: 716-724 https://doi.org/10.1079/9781845934132.0716

Pirgozliev V, Mansbridge SC, Rose SP, Lillehoj HS & Bravo D (2019). Immune modulation, growth performance, and nutrient retention in broiler chickens fed a blend of phytogenic feed additives. Poultry Science 98 (9): 3443-3449 https://doi.org/10.3382/ps/pey472

Pujiarti R, Ohtani Y & Widowati TB (2012). Kasmudjo Utilization of Melaleuca leucadendron essential oil. 2, 94-99.

Purnamasari L, Agus A & Noviandi CT (2020). Pengaruh Pemberian Asam Amino Metionin-Sistin pada Pakan yang Terkontaminasi Aflatoxin B1 Terhadap Mortalitas dan Kinerja Organ Dalam Ayam Broiler. Jurnal Ilmu Ternak, 20(1):46-55 https://doi.org/10.24198/jit.v20i1.28582

Qu H, Cheng Y, Chen Y, Zhao Y, Li J, Wen C & Zhou Y (2019). Dietary Tea Tree (Melaleuca alternifolia) Oil Supplementation Improves Growth Performance, Cecal Microflora, Immunity, and Antioxidant Capacity of Partridge Shank Chickens. The Journal of Poultry Science 56(3): 212-219. https://doi.org/10.2141%2Fjpsa.0180095

Ronquillo MG & Hernandez JCA (2017). Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods Food Control, 72:255-267 https://doi.org/10.1016/j.foodcont.2016.03.001

Sagar KS, Chang CC, Wang WK, Lin JY, Lee SS (2004). Preparation and anti HIV activities of retro justicidin B analogs and azalignans. Bioorganic & Medical Chemistry. 12: 4045–4054 https://doi.org/10.1016/j.bmc.2004.05.036

Sibiya AM, Ramya AK & Vaseeharan B (2020). Bioactive Molecules from Phyllanthus Niruri and Investigating their effects against Diabetes. Recent Trends in Biochemistry: MedDocs Publishers LLC. http://meddocsonline.org/

Siddique S, Parveen Z, Firdaus-E-bareen Mazhar S (2020). Chemical composition, antibacterial and antioxidant activities of essential oils from leaves of three Melaleuca species of Pakistani flora Arab. Journal of Chemistry., 13, 67-74 http://dx.doi.org/10.1016/j.arabjc.2017.01.018

Suhirman S & Winarti C (2010). Prospek dan fungsi tanaman obat sebagai imunomodulator. Bogor: Balai Penelitian Tanaman Obat dan Aromatik & Balai Besar Penelitian dan Pengembangan Pascapanen Pertanian.

Tabeekh MAS & Mayah AAS (2009). Morphological investigation of Bursa of Fabricius of imported broilers and local chicks vaccinated with two types of IBD vaccines. Iraqi Journal of Veterinary Sciences 23: 201

Talazadeh F & Mayahi M (2017). Immune response of broiler chickens supplemented with pediatric cough syrup including thyme extract in drinking water against influenza vaccine Journal of Herbmed. Pharmacology., 6 (1); 33-36

Than NN, Fotso S, Poeggeler B, Hardeland R & Laatsch H (2006). Niruriflavone, a new antioxidant flavone sulfonic acid from Phyllanthus niruri. Z. Naturforsch 61b: 57–60

Tolve R, Tchuen-Magaia F, Cairano MD, Caruso CC, Scarpa T & Galgano F (2021). Encapsulation of bioactive compounds for the formulation of functional animal feeds the biofortification of derivate foods. Animal Feed Science and Technology, 279 https://doi.org/10.1016/j.anifeedsci.2021.115036

Ukit U, Widiana A, Rahmawati E & Hasby RM (2019). Antibacterial activities test of Cajuput Leaf Waste extract (Melaleuca cajuputi Powell) on Pathogenic Bacteria. Journal of Physics: Conference Series https://doi.org/10.1088/1742-6596/1402/3/033030

Wardani AAK (2009). Pengaruh pemberian ekstrak etanol Temulawak (Curcuma xanthorriza Roxb.) terhadap gambaran histopatologi bursa Fabricius ayam petelur [skripsi]. Bogor: Fakultas Kedokteran Hewan, Institut Pertanian Bogor.

Widiana A, Taufikurahman, Suwido HL & Robert M (2015). The Potential Gelam Leaf as Nontimber Product of the Trees for Reforestation of the Degraded Peat land in Central Kalimantan Indonesia. Advances in Environmental Biology 9(2):13-17.

Zhang J, Wu H, Jiang D, Yang Y, Tang W & Xu K (2018). The antifungal activity of essential oil from Melaleuca leucadendron (L.) L. grown in China and its synergistic effects with conventional antibiotics against Candida. Natural Product Research https://doi.org/10.1080/14786419.2018.1448979

Zhao J, Liu TT & Chen G (2016). An effective β-cyclodextrin polyurethane spherical adsorbent for the chromatographic enrichment of corilagin from Phyllanthus niruri L. extract. Reactive and Functional Polymers. 102: 119-129 https://doi.org/10.1016/j.reactfunctpolym.2016.03.019

Zumbaugh CA, Murugesan GR, Wong EA, Syed B & Persia ME (2020). Evaluation of a phytogenic feed additive on performance, nutrient digestion, and absorption in turkey poults. Animal Feed Science and Technology, 267 https://doi.org/ 10.1016/j.anifeedsci.2020.114575