

## THE EFFECT OF JAMU FORMULAS ON BODY WEIGHT, ANTIOXIDANT ACTIVITIES, AND ANTIBODY TITER IN CHICKEN

### *Pengaruh Jenis Jamu terhadap Bobot Badan, Aktivitas Antioksidan dan Titer Antibodi Ayam*

BAGEM BR SEMBIRING<sup>1)</sup>, ENING WIEDOSARI<sup>2)</sup> dan SUJANTO<sup>1)</sup>

<sup>1)</sup> Indonesian Spices and Medicinal Crops Research Institute (ISMCRI)

<sup>2)</sup> Indonesian Research Center for Veterinary Sciences (IRCVS)

email: [anna.sembiring@yahoo.co.id](mailto:anna.sembiring@yahoo.co.id)

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#### ABSTRACT

Medicinal plants are useful to increase body immunity, body weight, appetite, and improve health both for human and animal. The research aimed to obtain functional drink formula for chicken, called jamu, made with spices and medicinal plants. These trials consisted of 3 activities; first, raw materials preparation include fermentation and extraction, second, functional drink formulation in form liquid and powder with pH and IC50 value as parameters, third, efficacy test of formulas that had the strongest antioxidant. The treatments were categorized as group I (chicken were fed with jamu, without vaccination), group II (fed with jamu for two weeks then vaccinated), group III (jamu feeding complemented by vaccination), group IV (control, only vaccination). The third activity was arranged in a randomized block design with 6 replications. Parameters observed were body weight increment and antibody titer. The fermentation time and formula composition showed no significant effect on pH until the third day (4.31 - 4.68), but they indicated significant effect on pH from the fourth day, declined until the seventh day (3.65-4.26). The type and compositions of the formula significantly affected IC50 value. The smallest of IC50 value of liquid and powder formulas were 7796.25 ppm and 244.57 ppm, respectively. The incremental body weight regarding liquid, powder, and control formulas were 365.55 g / week, 351.22 g / week, and 326.66 g / week, respectively. The highest antibody titer was at group III that had 4.50 (log<sub>2</sub>), whereas control was 3.30 (log<sub>2</sub>). The combination feeding, jamu formula and vaccine, was able to increase body weight increment and antibody titer on chicken.

Keywords: medicinal plants, functional drink, IC50, broilers, antibody titers

#### ABSTRAK

Tanaman obat digunakan untuk meningkatkan daya tahan tubuh, bobot badan, nafsu makan, mencegah penyakit, serta pemulihan kesehatan manusia dan hewan. Penelitian bertujuan mendapatkan formula minuman fungsional untuk ayam berbasis tanaman rempah dan obat. Kegiatan meliputi 3 aktivitas (1) Penanganan bahan baku, fermentasi dan ekstraksi (2) Formulasi minuman fungsional (cair dan serbuk) dengan parameter pH dan nilai IC50. (3) Uji efikasi formula cair dan serbuk yang menghasilkan aktivitas antioksidan terkuat. Perlakuan: kelompok I, ayam hanya diberi formula jamu selama dua minggu, kelompok II, ayam diberi formula jamu selama dua minggu sebelum vaksinasi, kelompok III, ayam divaksin sebelum diberi formula jamu selama dua minggu, kelompok IV, ayam hanya divaksinasi. Kegiatan (3) menggunakan Rancangan Acak Kelompok dengan 6 ulangan dan parameter pengamatan yaitu penambahan bobot badan dan titer antibodi. Waktu fermentasi dan komposisi formula tidak menunjukkan efek yang signifikan pada pH sampai hari ketiga (4,31-4,68), namun signifikan pada hari keempat ditandai dengan penurunan pH sampai hari ketujuh (3,65-4,26). Jenis dan komposisi formula secara

signifikan mempengaruhi nilai IC50. Nilai IC50 terkecil dari formula cair adalah 7796,25 ppm dan serbuk 244,57 ppm. Kenaikan berat badan dengan formula jamu cair yaitu 365,55 g / minggu, serbuk 351,22 g / minggu dan kontrol 326,66 g / minggu. Titer antibodi tertinggi adalah 4,50 (log<sub>2</sub>) yang ditunjukkan oleh kelompok III, sedangkan kontrol 3,30 (log<sub>2</sub>). Minuman fungsional (jamu) ditambah dengan vaksinasi mampu meningkatkan pertambahan berat badan dan titer antibodi ayam.

Kata kunci: Tanaman obat, minuman fungsional, IC50, ayam broiler, titer antibodi

#### INTRODUCTION

Organic poultry farming is becoming popular in many countries as a new paradigm adopting environmental and sustainable technologies. Chicken can be easily attacked by diseases such as avian influenza and coccidiosis, so they are necessary to defense against the diseases that are generally caused by viruses and protozoa. The mechanism of disease preventions aims at improving the immune system.

To keep the poultry remains in good condition, it is necessary to provide supplement (functional drink) based on antioxidants that are useful to increase endurance. Functional drinks are products that have beneficial protection or prevention, treatment of disease, the improvement of performance optimal body function, and slowing the aging process (Sampoerno and Fardiaz 2001). Functional drinks contain the nutrients such as antioxidant that needed by the body to neutralize free radicals and prevent damage of normal cells such as proteins and fats. The free radicals can cause an intrusion and cell metabolites through malfunctioning of DNA and proteins, causing mutations or cytotoxic and changes in enzyme activity, which can suppress the growth of the broilers (Kinanti 2011). The antioxidants boost immune function in preventing disease and health preserving (Sen et al. 2010; De la Fuente 2002). The synthetic chemical has been monitored intensively addressing to produce healthy food and ecological benefits (Zhang et al. 2013). Moreover, the continuous application of chemical-based antioxidants can leave residues, since one of alternative solutions is by using

medicinal plants. Numerous studies have demonstrated that they have benefits like anti-oxidative, antimicrobial efficacy, and improving the flavor of meat (Landy et al. 2011). The medicinal plants, in form feed additive, have a lot of properties which can be used as natural anti-biotic growth promoter in livestock (Liu et al. 2011), anti-pathogenic activities and probiotic stimulatory effect (Zhou et al. 2016).

Medicinal plants can be processed into various herbal products that are beneficial to human and livestock health, known as jamu. Jamu is an Indonesian formula heritage that has a global prospect not only for human but also for animal by supporting organic poultry system. According Adams et al. (2007), medicinal plants can increase endurance, prevent disease, heal and restore health. Many active compounds contained in the medicinal plants can serve as antioxidants which are important for health, neutralizing and destroying free radicals (Rafieian-Kopaei and Baradaran 2013). The strength of antioxidant activity is determined by the value of IC50 (inhibition concentration of 50%). According to Windono et al. (2001), the smaller the IC50 value of a substance, the stronger the antioxidant activity to capture free radicals.

Some of medicinal plants are important sources of natural antioxidants, such as ginger, turmeric, ashitaba, gotucole and blue ginger. Ginger extract can effectively inhibits microbial growth of *Bacillus alvae* and *Bacillus licheniformi* with inhibition zone of gram-positive measurement 24.5 mm and 15.25 mm, respectively (Purwani et al. 2001). It also effectively inhibits *Salmonella aereus* and *E. coli* with inhibition zone measuring 15.83 mm and 15.33 mm, respectively. The active compounds of ginger such as gingerol and shogaol have stronger antioxidant activity than vitamin E (Kikuzaki and Nakatani 1993). The active compounds of Javanese turmeric extract such as curcumin, xanthoxol and essential oils are beneficial as an antibacterial, immune-modulatory, appetite enhancer, improve heart function, and improve the appearance of blood lymphocytes (Septiana et al. 2006). Extracts of turmeric and ginger can increase the activity of the immune system (Septiana et al. 2006). Radiati et al. 2003 stated that consumption of ginger extracts in functional beverages and traditional medicines can increase their endurance and treat from diarrhoea. According to Zakaria et al. 1999, ginger extract can increase activity of Natural Killer (NK) Cells, induced body resistant to virus attacks because the cells is specifically capable of destroying infected cell by virus.

Ashitaba (*Angelica keiskei* Koidzumi) is an introduction species containing chlorophyll that has functions as an anti-microbial agent, increasing blood production, and improving the balance of body functions. In addition, it contains Chalcone and vitamin B12 substances that are useful to help a metabolism process, increasing production of hemoglobin, attention and concentration mind, growth hormone production, defense system against infectious diseases, and cancer (Zhang et al. 2013). According to Chen et al. (2004), toxicology

assessment of Ashitaba resulted that its chalcone powder was genotoxic with a NOAEL of 300 mg/kg in male and female rat (Maronpot 2015). Similar as the toxicological test of ashitaba's extract given to mice (*Mus musculus*) with dosage 125 to 1.000 mg, has no significant effect on liver of (Swarayana et al. 2012).

Blue ginger can be used as an anthelmintic due to its essential oil that causes the paralysis of the worm muscle (Widowati 2007). The combination of *curcumin* and essential oil contained in blue ginger extract and Javanese turmeric can kill the worms in the digestive tract of laying hens and improve their immune (Damayanti et al. 2009). Combination of Javanese turmeric extract and blue ginger with ratio 1:1 can reduce free radical about 94,43% (Rukayadi and Hwang 2013). According to Hidayana 2015, ethanol extract from blue ginger can increase the phagocytic ability and the number of white blood cells (WBC) that plays a key role in the body's immune system. Furthermore, gotucole (*Centella asiatica*) has a pharmacological effect for wound healing, inflammation, tuberculosis, dysentery, fever, and appetite enhancement (Roy et al. 2013). According to Syifaiah (2008), asiatic acid contained in *Centella asiatica* is beneficial for protecting the body from the influence of free radicals.

This study was aimed to obtain the effective jamu formula as poultry functional drinks based on antioxidants.

## MATERIALS AND METHODS

The experiment was conducted in the laboratory of Indonesian Spices and Medicinal Crops Research Institute (ISMCRI), and Veterinary Laboratory of Indonesian Research Center for Veterinary Science (IRCVS), Bogor, West Java in 2014. Plant material such as ashitaba and gotucole were obtained from experimental garden, Manoko Lembang, Bandung. The red ginger, javanese turmeric, and blue ginger were taken from Cicurug, Sukabumi, West Java. Day old chickens (DOCs), experimental animals, were used to evaluate the effectiveness of formula. The study consisted of the material preparation starting from extraction, fermentation, drying of extract, formulation, testing the formula antioxidant activity (*IC50*), and applications to the chickens. The observation parameters were pH, body weight, and antibody titers. The formulas consisted of two types; liquid and powder. Each type comprises three formulas (Table 1).

### Formulation

Liquid formula was made by mixing ashitaba, gotu kola, red ginger, turmeric and blue ginger into one determined composition. All raw materials and formulation were conducted in the fresh form. Subsequently, while the ingredients of each formula were blended, the distilled water was added as much as 10 liter/kg of the material. The obtained slurry was squeezed and filtered then the filtrate was placed in a bucket. Molasses and EM4 was added into

each formula and stirred until distributed evenly. Then the bucket was closed and fermented in one week. Observations were done every day by opening the lid, stirring the solution, checking pH value, reclosing the bucket and measured IC50.

The powder formula consisted of three formulas which containing ashitaba, gotu kola, red ginger, Javanese turmeric, and blue ginger. The formulas were made from decent mixture of fresh raw material. The raw material of each formula was weighed and then processed into a slurry form using a blender. The slurry obtained mixed with 70% ethanol and extracted for 4 hours. The filtrate was evaporated using a rotary evaporator to produce a viscous extract. Furthermore, viscous extract was processed into a dry extract, and then formulated due to the composition of the formula. Composition of formula was based on the previous study focused the testing of antioxidant activity of herbal drinks included the physical characteristic, organoleptic analysis and affixation test as immunomodulation on Webster female mice (Sembiring 2014). The parameters observed were *IC50* (*dpph method*) (Table 1).

### Cage and DOC preparations

The efficacy test of the formulas was conducted in veterinary laboratory of Indonesian Research Center for Veterinary Science. Preparation of the cage, sterilization, fumigation, sanitation, and providing drinking water were conducted before the DOCs arrived. The next stage was the preparation of DOCs and feed.

Table 1. The composition of a functional drink formula  
*Tabel 1. Komposisi formula minuman fungsional*

Treatment/Perlakuan	F1 (%)	F2 (%)	F3 (%)
Ashitaba/Ashitaba	20	30	40
Gotu kola/Pegagan	40	30	20
Red ginger/Jahe merah	20	15	10
Javanese turmeric/Temulawak	10	15	20
Blue ginger/Temu ireng	10	10	10
Total/Jumlah	100	100	100

Table 2. The applications of jamu formulas in chickens  
*Tabel 2. Aplikasi formula jamu terhadap ayam*

Group/ Kelompok	Formula type/ Bentuk formula	Jamu	Jamu application before vaccination/ Aplikasi jamu sebelum vaksin	Jamu application after vaccination/ Aplikasi jamu setelah vaksin	ND vaccine/ Vaksin ND
I	Liquid/Cair	√			
	Powder/Serbuk	√			
II	Liquid/Cair		√		
	Powder/Serbuk		√		
III	Liquid/Cair			√	
	Powder/Serbuk			√	
IV	Control (vaccine)/Vaksin				√

Note: Group I = Chickens given herbal formula for two weeks; Group II = Chicken was given herbal formula for two weeks before being vaccinated; Group III = Chicken is vaccinated, before being given a herbal formula for two weeks; Group IV = Chicken only be vaccinated

The daily base feed given were like conventional farmers, BR I crumble and G11 crumble for starter time, and BR II for 22<sup>th</sup> day-harvesting day.

*Keterangan: Kelompok I = Ayam diberi formula jamu selama dua minggu; Kelompok II = Ayam diberi formula jamu selama dua minggu sebelum divaksin; Kelompok III = Ayam divaksin, sebelum diberi formula jamu selama dua minggu; Kelompok IV = Ayam hanya divaksinasi Pakan pokok yang diberikan seperti yang petani secara konvensional lakukan BR1 crumble, G11 crumble untuk periode starter, dan BR II untuk hari ke-22 hingga panen.*

Preparation of DOCs was carry out by sorting chicks based on average body weight (37-40 g/head). One hundred of selected DOCs were kept without treatment. After looking after them for one week, chickens were selected as many as 15 for each treatment. Every week the antibody titer was checked and it had performed for three weeks. At three weeks of age it was estimated that the antibody titer of chicks was close to zero.

### Formula Efficacy

Efficacy test was conducted on the formulas which produce the strongest antioxidant activity that shown by the smallest IC50 value. The 48 head of chicken had been used and kept for 3 month. Treatment was divided into four groups and each consisting of 12 chickens (Table 2). Giving of formulas was carried out by in-feeding liquid formula and powder formula to 6 head of chickens, respectively. Each treatment unit consisted of 6 broiler chickens (Table 2) and placed one by one in a cage. This was done in the same way as in each group, except group IV. In that group, chickens were not given jamu, but given ND vaccine only. For the formula in the form of dry extract, it had to be dissolved with water before applied. Before and after application, the chickens were weighed and taken blood to check for antibody titers.

The observations had been conducted for three weeks after application. This research used random group design (RGD) with observation parameters: weight gain, antibody titers, and the percentage of mortality.

For evaluating the serum HI (*Haemagglutination inhibition*) antibody assay (OIE 1991), Blood samples (1.5 ml per chick) were drawn into *ependorf* tubes from the main brachial vein and allowed to clot at 37°C for 2 h prior to be collected its serum. Serum was separated by centrifugation and stored at a temperature -20°C. Briefly, two fold serial dilution of serum were made in a 96-well, V shaped bottom micro titer plate containing 0.025 ml of PBS in all wells and then 0.025 ml of NDV antigen (4 HA units) was added into all the wells except for the last row served as the controls. The antigen serum mixture was incubated for 30 min at room temperature. Then 0.025 ml of a 1% chicken erythrocytes suspension was added to each well and re-incubated for 40 min. A positive serum and a negative serum were included as controls. The highest dilution of serum causing complete inhibition was considered the end point. The geometric mean titer was expressed as reciprocal log2 values of the highest dilution that displayed HI.

the same. The result of formula fermentation until the third day obtained pH levels range from 4.31 to 4.68. According to statistical analysis, pH values between the three formulas were not significantly different ( $P > 0.05$ ). The composition and period of fermentation did not affect to the pH value of the formula. The pH value of the formula began to decrease on the 4th day of fermentation. The higher acidity of the formula was indicated by the pH value decreased to 3.64-4.34. The results of statistical analysis showed that the pH values of F1, F2, and F3 at the 5% level were not significantly. The composition and fermentation time of fourth day to seventh day affected to the pH value of F1 and F2, except F3. The pH value of seventh day fermentation of F1 and F2 were 3,65 and 4,26 F3, respectively. According to Shrestha et al. (2012) the longer the fermentation time, the more acidity formed and indicated by decreasing pH level. Fermentation length has an effect on pH level and antioxidant activity (Primurdia and Kusnadi 2014). According to Azizah et al. (2012), the factors that affect the fermentation process are the substrate, temperature, pH, oxygen, and microbes. Moreover in the previous research conducted by Steinkraus (2002), the kombucha drink consumed should have a pH value between 2.5 and 4.6.

RESULTS AND DISCUSSION

The formula of Liquid Jamu

pH (*Potentia Hydrogenii*) Value

The compositions of the materials among three formulas were different, but the fermentation times were

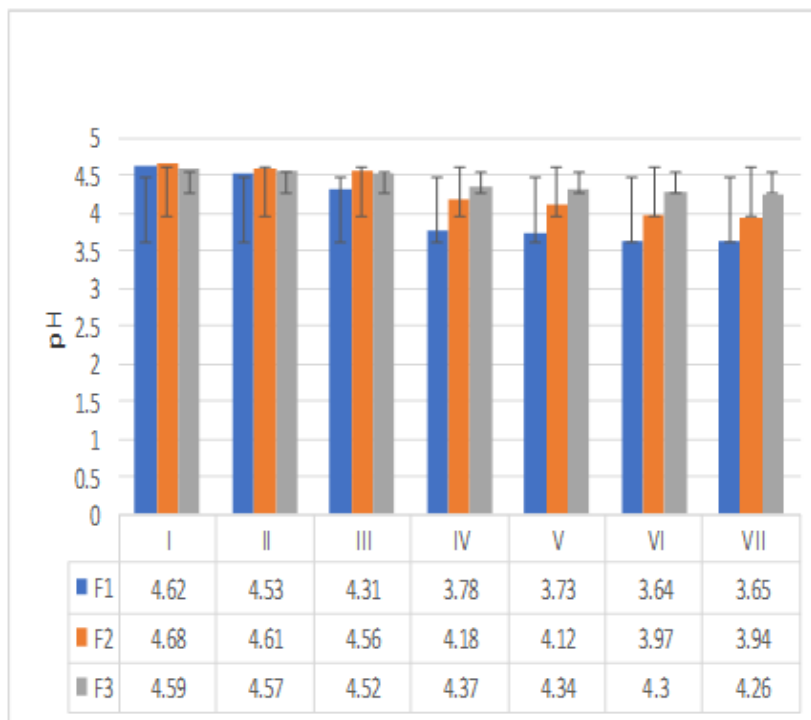


Figure 1. The pH value of liquid formula on the time of fermentation 1 to 7 days.  
 Gambar 1. Nilai pH formula cair pada lama fermentasi 1-7 hari

Formula 1= Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (20:40:20:10:10)  
 Formula 2 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (30:30:15:15:10)  
 Formula 3 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (40:20:10:20:10)

The first formula has a major composition of gotu kola (40%), the second formula contained ashitaba and gotu kola with the same amount (30%:30%), and the third formula comprised of ashitaba 40%. The ashitaba contains flavonoid compounds that are more useful as an antioxidant than grapes, soy bean, and green tea. In addition, the antioxidant activity of ashitaba is stronger than gotu kola (Sembiring and Manoi 2015). The active compound contained in ashitaba can maintain the pH value during the fermentation process.

## Antioxidant Activities

### Liquid Formula of Jamu

The value of (IC<sub>50</sub>) of liquid jamu had range of 7796.25-9422.50 ppm. The results showed that the third formula had stronger antioxidant activity than formula 1 and 2. F3 had IC<sub>50</sub> values smaller than others formula. The result of advance Duncan test at level of significance 5% showed that IC<sub>50</sub> value of F1 and F2 had a same superscript letter. The antioxidant activities of F1 and F2 were not significantly different (P>0,05). The F2 and F3 had a significant level (P<0.05). The composition of the formula does not significantly affect the IC<sub>50</sub> value, except for formula 3. In third formula, ashitaba contains as much as 40% and the amount was greater than first and second formula. The more the amount of ashitaba added to the formula, the more potential of the active compound as an antioxidant. Thus the use of liquid formula three was more effective, because the dosage of F3 at 7796.25 ppm already inhibited free radicals by 50% (Table 3). According to Suhardini and Zubaidah (2016), acidity factor also affects antioxidant activity. In acidic state the phenolic compounds are unstable and difficult to release protons which can bind with so DPPH, therefore the antioxidant activity decreases.

According to Windono et al. (2001), the more less the IC<sub>50</sub> value of formula tested, the better its antioxidant activity capturing free radicals. The fermentation time affected to level of antioxidant activity whereas the longer time would be more power contained. The all formulas had been fermented for 7 days, but over fermentation time was not recommended because it could lead to accumulation of organic acid compound which was able to reach harmful level and could be dangerous for consumption (Srihari and Satyanarayana 2012; Primurdia and Kusnadi 2014).

Table 3. Antioxidant activities of liquid jamu's formula

Tabel 3. Aktivitas antioksidan formula jamu cair

Formula/Formula	Antioxidant activities (IC <sub>50</sub> %)/ Aktivitas antioksidan
F1	9422.50 ppm <sup>a</sup>
F2	9235.00 ppm <sup>a</sup>
F3	7796.25 ppm <sup>b</sup>

Note: Numbers followed by the same letter in the same column are not significantly different at the Duncan (test level of 5%).

Keterangan: Angka yang diikuti huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf uji Duncan 5%

Formula 1 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (20:40:20:10:10)

Formula 2 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (30:30:15:15:10)

Formula 3 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (40:20:10:20:10)

### Powder Formula of Jamu

The IC<sub>50</sub> values of the first, second and third formula were 255.51 ppm, 252.17 ppm and 244.57 ppm respectively. The high concentration of ashitaba contained in formula, resulting smaller IC<sub>50</sub> value and vice versa. The IC<sub>50</sub> values of formula 1 and 2 were significantly different from third formula. The antioxidant activity of third was stronger than formula 1 and 2, it might because of higher ashitaba concentration. Moreover, the individual IC<sub>50</sub> value of ashitaba and gotucola are about 38 ppm, and 44.91 ppm, respectively. According to Caesar and Cech (2016) the flavonoid compounds contained in ashitaba have a role as an antioxidant.

Table 4. Antioxidant activities of powder Jamu formula

Tabel 4. Aktivitas antioksidan formula jamu serbuk

Formula	Antioxidant activities (IC <sub>50</sub> )/ Aktivitas antioksidan
F1	255,51 ppm <sup>a</sup>
F2	252,17 ppm <sup>a</sup>
F3	244,57 ppm <sup>b</sup>

Note: Numbers followed by the same letter in the same column are not significantly different at the Duncan test (level of 5%).

Keterangan: Angka yang diikuti huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf uji Duncan 5%.

Formula 1 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (20:40:20:10:10)

Formula 2 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (30:30:15:15:10)

Formula 3 = Ashitaba, gotu kola, red ginger, Javanese turmeric, blue ginger (40:20:10:20:10)

## Efficacy Test of Jamu Formulas

### Body Weight Gain

The tests results of IC<sub>50</sub> values from third formula resulted the strongest antioxidant activity shown by the smallest IC<sub>50</sub> value. The liquid and powder of third formula had an IC<sub>50</sub> value of 7796.25 ppm and 244.57 ppm, respectively. Both types of formula were tested to the chicken to know their effectiveness as a functional drink. The result of application of liquid formula in group I, II, and III resulted in the average body weight of chicken were 331.66, g/head/week, 365.55 g/head/week, and 293.33 g/head/week, respectively. Whether the powder formulas I, II, and III application obtained mean of increasing body weight 351.11 g/head/week, 340.55 g/head/week, and 345.55 g/head/week, respectively. Whereas the chicken body weight mean of the fourth group was 326.66 g/tail/week. The increasing mean weights of chickens that treated by jamu formula were not significantly different with chicken that given by vaccine. Statistical analysis showed that mean of body weight gain of the liquid formula in group II and the liquid formula in group III was significant at level 5%. It is accordance with significant different of antioxidant activity test between formula II and III. IC<sub>50</sub> of liquid formula II and III were 9,235 ppm and 7,796 ppm, respectively.

Table 4. Effect of application of liquid and powder formulas herbal against weight gain Chicken  
 Tabel 4. Pengaruh aplikasi formula jamu cair dan serbuk terhadap pertambahan bobot badan Ayam

Group/ Kelompok	Type of herbal / Jenis Jamu	Bobot Badan Ayam/minggu (g)/ Chicken body weight/week (g)				Average body weight gain/(g)/Penambahan bobot badan rata-rata (g)
		1	2	3	4	
I	Liquid/Cair	646,66	971,66	1301,66	1641,66	331,66 <sup>ab</sup>
	Powder/Serbuk	625,00	928,33	1265,00	1678,33	351,10 <sup>ab</sup>
II	Liquid/Cair	588,33	908,33	1280,00	1685,00	365,55 <sup>a</sup>
	Powder/Serbuk	566,66	855,00	1208,33	1588,33	340,55 <sup>ab</sup>
III	Liquid/Cair	583,33	821,66	1131,66	1463,33	293,33 <sup>b</sup>
	Powder/Serbuk	535,00	880,00	1250,00	1571,66	351,22 <sup>ab</sup>
IV	Vaccine/Vaksin	526,66	833,33	1113,33	1506,66	326,66 <sup>ab</sup>

Note: Numbers followed by the same letter in the same column are not significantly different at the Duncan test (level of 5%).

Keterangan: Angka yang diikuti huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf uji Duncan 5%.

Note: Group I = Chicken only given herbs for two weeks  
 Group II = Chicken was given herbs for two weeks before being vaccinated  
 Group III = Chicken was vaccinated before being given herbs for two weeks  
 Group IV = Chicken only be vaccinated

Keterangan: Kelompok I = Ayam hanya diberi jamu selama dua minggu  
 Kelompok II = Ayam diberi jamu selama dua minggu sebelum divaksin  
 Kelompok III = Ayam divaksin sebelum diberi jamu selama dua minggu  
 Kelompok IV = Ayam hanya divaksinasi

Everybody has endurance, if the amount of free radicals is too much, and the endogenous antioxidants contained in the body is inadequate, they will damage cells of the body (Sibuea in Sembiring and Manoi 2015). The antioxidants have an important role for health especially in defending the body from cell damage due to free radical element. According to Takashi And Takayuni in Zuhra et al. (2008), the synthetic antioxidant, BHT (Butylated Hydroxy Toluene), can be toxic and carcinogenic on experimental animals; therefore it needs an alternative to change to natural antioxidants. Ashitaba and temulawak contained in the formulas can function to accelerate the growth, extend the life of cells, and as an antibacterial agent, while the blue ginger serve as an anthelmintic. Wahyuni and Wakradiharja (2001), stated that mixed ginger, blue ginger and mojo fruit and gave them to poultry for improving productivity and preventing from AI (*avian influenza*) infection. The formula that contained king of bitter, red ginger, turmeric Javanese, and blue ginggaer can increase daily weight gain of chicken (Wiedosari et al. 2016).

Javanese turmeric extract contains curcumin consisting of desmetoxicurcumin and bisdesmetoxicurcumin and also the essential oil containing xanthorrhizol. The Java turmeric can be beneficial to boost immunity and

appetite, improve heart function, and improve the performance of blood lymphocytes (Septiana et al. (2006a)).

**Antibody titers (Haemagglutination inhibition)**

The observation of the chicken antibody titer indicated that the average of antibody titer at 2 weeks old before being given herbal was 4.96 (*log 2*) and the following week dropped to 2.77 (*log 2*). However, in the first week after being given herbs and ND vaccine, antibody titers of groups (I, II and III) higher than in group IV (only vaccinated). In the third week of antibody titer increased but only in group III, was significantly higher than in group IV and other treatment groups (P<0.05). Ronohardjo (1980), said the chicken antibody titer can reach the maximum within 14-25 days after vaccination and then it will decline continually.

Serum antibody titer is the indicator of humoral immunity. This experiment results showed that the antibody titers only in the third group at each time point were higher than that from control group. Suggesting that they could promote humoral immunity and that the maintenance time of their effects was the longest. The third formula promote the production of antibody in the immunosuppressant animals and could enhance the antibody titer of ND vaccine.



Figure 2. Application of jamu in chickens  
 Gambar 2. Aplikasi jamu pada ayam

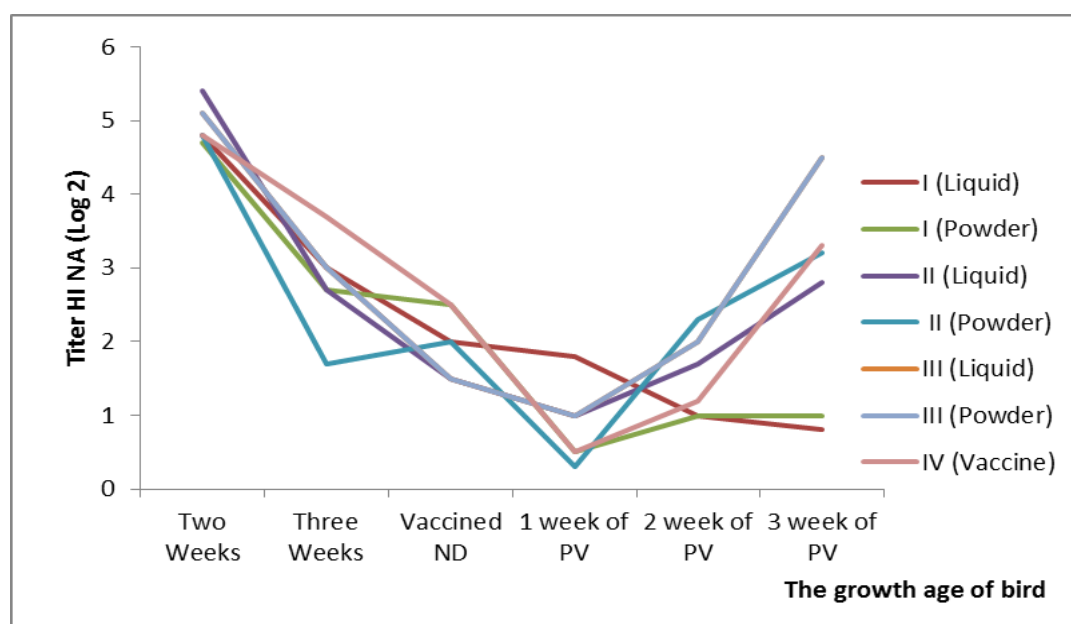


Figure 3. Total average antibody titers before given herbal formula  
 Gambar 3. Jumlah titer antibodi rata-rata sebelum diberi formula jamu

Note: Group I = Chicken only given herbs for two weeks  
 Group II = Chicken was given herbs for two weeks before being vaccinated  
 Group III = Chicken was vaccinated before being given herbs for two weeks  
 Group IV = Chicken only be vaccinated

Keterangan: Kelompok I = Ayam hanya diberi jamu selama dua minggu  
 Kelompok II = Ayam diberi jamu selama dua minggu sebelum divaksin  
 Kelompok III = Ayam divaksin sebelum diberi jamu selama dua minggu  
 Kelompok IV = Ayam hanya divaksinasi

The highest number of antibody titers of chickens were in group III with an average of 4.20 to 4.50 (log<sub>2</sub>) and the smallest antibody titers are treatment by group I with an average of 0.83-1.00 (log<sub>2</sub>), while group IV (control) is 3.30 (log<sub>2</sub>). This type of formula has no effect on the number of antibody titers, but the treatment in the group gives a significant effect. Statistical analysis showed that the number of chicken antibody titer in group III was significantly different with group I at level of significant 5%.

The vaccination with drinking water and jamu either in the form of liquid formula or powder can increase the antibody titer of chicken. Herbal formula used to test the efficacy of chicken antibody titers contain ashitaba as much as 40%, gotucola 20%, red ginger 10%, Java turmeric 20% dan blue ginger 10%. According to Sembiring and Manoi (2011), ashitaba extract has antioxidant activity and serves to keep the organs from free radicals damage. Both B6 vitamin and chlorophyll contained in ashitaba can stimulate appetite, growth and encouraging the culture of microorganism in the digestion system. Wicaksono and Syafirudin (2003) showed that ashitaba contains high

enough chlorophyll that can increase blood production. It also can improve the body's defence system against infectious diseases and cancer (Hida in Sembiring and Manoi 2015). Ashitaba leaves contain iron (Fe) as much as 435 ppm (Sembiring and Manoi 2011). In addition, the chalcone compounds contained in ashitaba is able to clean the blood, stimulates the liver function in neutralizing toxins, and improves kidney function in removing toxins from the blood efficiently (Inamori et al. 1991). According to the research conducted by Kimie Baba from Osaka University of Pharmacy, Japan (2009), chalcone compound is a flavonoid namely xantoangeol and 4-hidrooxyricine. Gotucola has a stimulatory effect on cellular antioxidant and an immune system that is used as a prophylactic against several diseases such as cardiovascular and stress (Shetty et al. 2008). The uses of extracts of ginger, garlic, turmeric and galangal are able to improve the immune system of experimental animals (Spelman et al. 2006). According to Winarsi (2005), the foods containing antioxidants can improve immunological status and inhibit the emergence of degenerative diseases.

Table 8. The effect of application liquid and powder jamu formulas on the chicken's antibody titer (HI)  
 Tabel 8. Pengaruh pemberian formula jamu cair dan serbuk terhadap titer antibodi (HI) Ayam

Group/Kelompok	Formulas type/ Jenis Formula	Prior to application/ Sebelum aplikasi	Total antibody titers (HI) log <sub>2</sub> / Jumlah titer antibodi/minggu			Average antibody titer (HI) log <sub>2</sub> / week /Titer antibodi rata- rata/minggu
			1	2	3	
I	Liquid/Cair	2,00	1,17	1,00	0,83	1 <sup>b</sup>
	Powder/Serbuk	2,50	0,50	1,00	1,00	0,833 <sup>b</sup>
II	Liquid/Cair	1,50	1,00	1,67	2,83	1,83 <sup>ab</sup>
	Powder/Serbuk	2,00	0,33c	2,33	3,17	1,943 <sup>ab</sup>
III	Liquid/Cair	1,50	1,00	2,00	4,50	2,5 <sup>a</sup>
	Powder/Serbuk	3,00	1,20	2,70	4,20	2,7 <sup>a</sup>
IV	Control (Vaccine ND)/Vaksin	2,50	0,50	1,20	3,30	1,67 <sup>ab</sup>

Note: Numbers followed by the same letter in the same column are not significantly different at the Duncan test (level of 5%).  
 Keterangan: Angka yang diikuti huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf uji Duncan 5%.

Note: Group I = Chicken only given herbs for two weeks  
 Group II = Chicken was given herbs for two weeks before being vaccinated  
 Group III = Chicken was vaccinated before being given herbs for two weeks  
 Group IV = Chicken only be vaccinated

Keterangan: Kelompok I = Ayam hanya diberi jamu selama dua minggu  
 Kelompok II = Ayam diberi jamu selama dua minggu sebelum divaksin  
 Kelompok III = Ayam divaksin sebelum diberi jamu selama dua minggu  
 Kelompok IV = Ayam hanya divaksinasi

One of the attempts to prevent chicken from Newcastle Disease (ND) virus infection is through vaccination. The success of vaccinations can trigger an antibody working optimal. The active compounds contained in the formula as antioxidants can be useful as immunomodulators. To obtain maximum results, the dosage of test formulas is made several levels so that the use of vaccine can be reduced.

CONCLUSION

The fermentation time and formula composition showed no significant effect on pH until the third day (4.31 - 4.68), but they indicated effect on pH from the fourth day, declined until the seventh day (3.65-4.26). The type compositions of the formula significantly affected IC50 value. The smallest of IC50 value of liquid and powder formulas were in formula III with IC50 value 7796.25 ppm and 244.57 ppm, respectively. The mean of chicken's body weight gain given jamu formula had significant different between liquid formula II, and liquid formula III with the value 365.55 g/head/week, and 293.33 g/head/week, respectively, but were not significant with the formula IV (control) with the value 326.66 g/head/week. The essential finding is Jamu formula from ashitaba, gotucola, red ginger, turmeric and blue ginger that made were not significantly different with the vaccine application due to incremental mean of body gain. It can be said that jamu can replace the vaccine application. Moreover, the highest average antibody titer (HI) was at liquid formula of group III 2.7 (log 2)/weak and not significantly different control 1.67 (log 2)/weak.

REFERENCES

Adams, M., Gmünder, F. & Hamburger, M. (2007) Plants traditionally used in age related brain disorders. A survey of ethnobotanical literature. *Journal of Ethnopharmacology*. 113 (3), Elsevier, 363-381.

Azizah, N., Al-Barrii, A.N. & Mulyani, S. (2012) Pengaruh lama fermentasi terhadap kadar alkohol, pH, dan produksi gas pada proses fermentasi bioetanol dari whey dengan substitusi kulit nanas. *Jurnal Aplikasi Teknologi Pangan*. 1 (3).

Caesar, L.K. & Cech, N.B. (2016) A Review of the medicinal uses and pharmacology of Ashitaba. *Planta Medica*. 82 (14), Georg Thieme Verlag KG, 1236-1245.

Chen, I.-C., Chang, H.-C., Yang, H.-W. & Chen, G.-L. (2004) Evaluation of total antioxidant activity of several popular vegetables and Chinese herbs: a fast approach with ABTS/H<sub>2</sub>O<sub>2</sub>/HRP system in microplates. *Journal of Food and Drug Analysis*. 12 (1).

Damayanti, E., Sofyan, A., Julendra, H. & Untari, T. (2009) Pemanfaatan tepung cacing tanah *Lumbricus rubellus* sebagai agensia anti-pullorum dalam imbuhan pakan ayam broiler. *JITV*. 14 (2), 83-89.

Hidayana, V.V. (2015) Pengaruh pemberian ekstrak etanol rimpang temu hitam (*Curcuma aeruginosa* Roxb.) terhadap kemampuan fagositosis dan jumlah sel leukosit pada mencit putih. *Jurnal Kesehatan STIKes Prima Nusantara Bukittinggi*. 5 (2).

Inamori, Y., Nishigushi, K., Matsuo, N., Ujibo, H. & Ishida, N. (1991) Phytogrowth-inhibitory activities of tropolone and hinokitiol. *Chemical and*



- pharmaceutical bulletin*. 39 (9), The Pharmaceutical Society of Japan, 2378-2381.
- Kikuzaki, H. & Nakatani, N. (1993) Antioxidant effects of some ginger constituents. *Journal of Food Science*. 58 (6), Wiley Online Library, 1407-1410.
- Kinanti, A.S. (2011) *Pengaruh suplementasi vitamin E dan DL- Methionine dalam ransum terhadap performa ayam broiler pada kondisi cekaman panas*. Institute Pertanian Bogor.
- De la Fuente, M. (2002) Effects of antioxidants on immune system ageing. *European Journal of Clinical Nutrition*. Doi:10.1038/sj.ejcn.1601476.
- Landy, N., Ghalamkari, G. & Toghyani, M. (2011) *Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (Azadirachta indica) as alternative for an antibiotic growth promoter*. *Livestock Science*. 142 (1). Doi:10.1016/j.livsci.2011.08.017.
- Liu, H., Tong, J. & Zhou, D. (2011) Utilization of chinese herbal feed additives in animal production. *Agricultural Sciences in China*. 10 (8), 1262-1272. Doi:10.1016/S1671-2927(11)60118-1.
- Maronpot, R.R. (2015) Toxicological assessment of Ashitaba Chalcone. *Food and Chemical Toxicology*. [Online] 77, 111–119. Doi:10.1016/j.fct.2014.12.021.
- OIE. 1991. Manual Of Standards For Diagnostic Tests And Vaccines for Lists A and B diseases of mammals, birds and bees. Office International Des Epizooties. Available online on <http://www.oie.int/doc/ged/D7722.PDF>.
- Primurdia, E.G. & Kusnadi, J. (2014a) Aktivitas antioksidan minuman probiotik sari kurma (*Phoenix dactylifera* L.) dengan isolat *L. Plantarum* dan *L. casei*. *Jurnal Pangan dan Agroindustri Vol. 2*. 2 (3), 98-109.
- Primurdia, E.G. & Kusnadi, J. (2014b) Aktivitas Antioksidan Minuman Probiotik Sari Kurma (*Phoenix dactylifera* L.) dengan isolat *L. Plantarum* dan *L. casei* [IN PRESS JULI 2014]. *Jurnal Pangan dan Agroindustri*. 2 (3), 98-109.
- Purwani, E., Wulang, S. & Hapsari, N. (2001) Respon hambatan bakteri gram positif dan negatif pada ikan nila (*Oreochromis niloticus*) yang diawetkan dengan ekstrak jahe (*Zingiber officinale*). *Jurnal Kesehatan*. 2 (1), 61-70.
- Radiati, L., Nabet, P., B., N., Capiaumont, J., Fardiaz, D., Zakaria, R.F., Sudirman, I. & Hariyadi, R. (2003) Pengaruh ekstrak diklorometan jahe (*Zingiber officinale* Roscoe) terhadap pengikatan toksin kolera B-subunit conjugasi (FITC) pada receptor sel Hibridoma LV dan Caco-2. *Jurnal Teknologi dan Industri Pangan*. XIV (1), 59-67.
- Rafieian-Kopaei, M. & Baradaran, A. (2013) Oxidative stress and the paradoxical effects of antioxidants. *Journal of Research in*. [Online] (July), 2970.
- Ronohardjo, P. (1980) *Beberapa Masalah yang Menyangkut Pengendalian Penyakit Tetelo (Newcastle Disease) di Indonesia*. In: *Makalah Seminar Penyakit Reproduksi dan Unggas. LPPH. Bogor*. pp.127-141.
- Roy, D.C., Barman, S.K. & Shaik, M.M. (2013) Current updates on *Centella asiatica*: phytochemistry, pharmacology and traditional uses. *Medicinal Plant Research*. 3.
- Rukayadi, Y. & Hwang, J. (2013) In vitro activity of xanthorrhizol isolated from the rhizome of Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) against *Candida albicans* biofilms. *Phytotherapy Research*. 27 (7). 1061-1066.
- Sampoerno dan D. Fardiaz (2001) *Kebijakan dan Pengembangan Pangan Fungsional dan Suplemen di Indonesia*. Pusat Kajian Makanan Tradisional, Institut Pertanian Bogor, pp.1-15.
- Sembiring, B.B. & Manoi, F. (2011) Identifikasi mutu tanaman Ashitaba. *Buletin Penelitian Tanaman Rempah dan Obat*. 22 (2). (2), 177-185.
- Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y.S.R. & De, B. (2010) Free radicals, antioxidants, diseases and phytomedicines: current and future prospect nitrogen species. *International Journal of Pharmaceutical Sciences Review and Research*. 3 (1), 95-96.
- Septiana, A.T., Dwiyantri, H., Muchtadi, D. & Zakaria, F. (2006a) Penghambatan Oksidasi LDL dan Akumulasi Kolesterol pada makrofag oleh ekstrak temulawak (*Curcuma xanthorrhiza* Roxb). *Jurnal Tekn dan Industri Pangan*. 17 (3), 221-226.
- Septiana, A.T., Dwiyantri, H., Muchtadi, D. & Zakaria, F.R. (2006b) Penghambatan oksidasi LDL dan akumulasi kolesterol pada makrofag oleh ekstrak temulawak (*Curcuma xanthorrhiza* Roxb). IPB (Bogor Agricultural University).
- Shetty, B.S., Udupa, S.L. & Udupa, A.L. (2008) Biochemical analysis of granulation tissue in steroid and *Centella asiatica* (Linn) treated rats. *Pharmacologyonline*. 2, 624-632.
- Shrestha, A.K., Arcot, J. & Yuliani, S. (2012) Susceptibility of 5-methyltetrahydrofolic acid to heat and microencapsulation to enhance its stability during extrusion processing. *Food chemistry*. 130 (2), Elsevier, 291-298.
- Spelman, K., Burns, J.J., Nichols, D., Winters, N., Ottersberg, S. & Tenborg, M. (2006) Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. *Alternative Medicine Review*. 11 (2), 128-150.
- Srihari, T. & Satyanarayana, U. (2012) Changes in Free radical scavenging activity of kombucha during fermentation. *Journal of Pharmaceutical Sciences and Research*. 4 (11), 1978-1981.
- Steinkraus, K.H. (2002) Fermentations in world food processing. *Comprehensive Reviews in Food Science and Food Safety*. 1 (1), Wiley Online Library, 23-32.

- Suhardini, P.N. & Zubaidah, E. (2016) Studi aktivitas antioksidan Kombucha dari berbagai jenis daun selama fermentasi. *Jurnal Pangan dan Agro Industri*. 4 (1), 221-229.
- Swarayana, I Made, I., Sudira, I.W. & Berata, I.K. (2012) Perubahan histopatologi hati mencit (*Mus musculus*) yang diberikan ekstrak daun *Ashitaba* (*Angelica keiskei*). *Buletin Veteriner Udayana*. 4 (2), 119-125.
- Syifaiah, B. (2008) Pengaruh ekstrak daun pegagan (*Centella asiatica*) terhadap kadar SGPT dan SGOT hati mencit (*Mus musculus*) yang diinduksi parasetamol. Universitas Islam Negeri Maulana Malik Ibrahim.
- Wahyuni, Z. & Wakradiharja, E. (2001) Racikan ramuan tanaman obat dalam bentuk larutan jamu dapat meningkatkan kesehatan hewan serta produktifitas ternak ayam buras. In: *Seminar Nasional Tumbuhan dan Obat Indonesia XIX*.
- Wicaksono, R. & Syafirudin, H. (2003) *Ashitaba* (*Angelica keiskei* Koidzumi) tanaman peningkat sistem kekebalan tubuh. In: *Prosiding Seminar dan Pameran Nasional Tumbuhan Obat Indonesia XXIV*. hlm. pp.270-275.
- Widowati, W. (2007) Peran Antioksidan sebagai agen hipoklesterolemia. *Majalah Kedokteran Damianus*. 6 (3), 228-230.
- Wiedosari, E., Suhirman, S. & Sembiring, B.B.R. (2016) Pengaruh jamu herbal sebagai antikoksidia pada ayam pedaging yang diinfeksi *Eimeria tenella*. *Jurnal Penelitian Tanaman Industri*. 20 (1), 9-16.
- Winarsi, H. (2005) *Antioksidan Alami dan Radikal*. Kanisius.
- Windono, T., Hendrajaya, K., Nurfatmawati, H. & Soraya, F. (2001) Uji perendaman radikal bebas terhadap DPPH dari ekstrak kulit buah dan biji anggur (*Vitis linifera*) Probolinggo Biru dan Bali. *Artikel hasil penelitian Artocarpus*. 1, 34-43.
- Zakaria, F., Wiguna, Y. & Hartoyo, A. (1999) Konsumsi sari jahe (*Zingiber officinale* Roscoe) meningkatkan sel natural killer pada mahasiswa pesantren Ulil Albaab di Bogor. *Buletin Tehnologi Industri Pangan*. 10 (2), 40-6.
- Zhang, E.H., Wang, R.F., Guo, S.Z. & Liu, B. (2013) An update on antitumor activity of naturally occurring chalcones. *Evidence-Based Complementary and Alternative Medicine*. 1-22. Available from: Doi:10.1155/2013/815621.
- Zhou, Q., Wang, S., Yang, G., Zhao, W. & Li, H. (2016) Development and evaluation of a herbal formulation with anti-pathogenic activities and probiotics stimulatory effects. *Journal of Integrative Agriculture*. 15 (5), 1103-1111. Doi:10.1016/S2095-3119(15) 61146-7.
- Zuhra, C.F., Tarigan, J.B. & Sihotang, H. (2008) Aktivitas Antioksidan senyawa flavonoid dari daun katuk (*Sauropus androgynus* (L.) Merr.). *Jurnal Biologi Sumatra*. 3 (1), 7-10.