PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 14 (8): 492-497, 2015
ISSN 1680-5194

Content of Phytochemical Compound and Antibacterial Activity of Cinnamon Leaf (*Cinnamomum burmanii*) and Noni Fruit and Leaf (*Morinda citrifolia* L) Mixture Extract to Replace Antibiotics

A. Yuniza and Yuherman
Faculty of Animal Husbandry, Andalas University, Padang, West Sumatra, Indonesia

Abstract: This research was conducted to determine the best extraction method to produce cinnamon leaf (*Cinnamomum burmanii*) and noni fruit and leaf (*Morinda citrifolia* L) mixture extract as source of phytochemical compound to replace the role of antibiotic in broiler production. The mixture extract was named as 'Cinnamononi extract'. There were four different extraction methods in these experiments, i.e. type A: maceration extraction method with aquadest solvent, type B: maceration extraction method with methanol solvent, type C: modified of reflux extraction and type D: combination of reflux and maceration extract. Two experiments were conducted to evaluate phytochemical compound of four types of cinnamononi extract and to examine antibacterial activity in these extracts. The antibacterial activity of these extracts on *Escherichia coli* and *Salmonella typhimurium* were determined using agar ditch diffusion method. The experiment 2 was designed as completely randomized design (CRD) with 5 times replications. There were 9 treatments in this experiment, i.e.: T = antibiotic tetracycline 0.02 g/ml, A1 = cinnamononi extract type A with dilution concentration 1 g/ml, A2 = type A, concentration 0.1 g/ml, B1 = type B, concentration 1 g/ml, B2 = type B, concentration 0.1 g/ml, C1 = type C, concentration 1 g/ml, C2 = type C, concentration 0.1 g/ml, D1 = type D, concentration 1 g/ml, D2 = type D, concentration 0.1 g/ml. Variable in this experiment was inhibition diameter of zone (clear zone) produced after incubation. The result of experiment 1 showed that strong compound containing cinnamononi extract type A, C and D were phenolic (+++), whereas type B was triterpenoid (+++) and negative flavonoid (-). The result of experiment 2 showed that treatments have highly significant effect ($p<0.01$) to zone of inhibition of *E. coli* and *Salmonella* sp. Antibacterial activity of all cinnamononi extract with concentration 0.1 g/ml could replace the role of tetracycline to inhibit *Salmonella* sp, but to inhibit *Escherichia coli* higher concentration was needed, i.e., 1 g/ml. In conclusion, type A and C of cinnamononi extract had the best activity to inhibit *Escherichia coli* bacterial, but only type C of cinnamononi extract which have the best activity to inhibit *Salmonella* sp.

Key words: Antibacterial activity, mixture extract, phytochemical compound, *Cinnamomum burmanii*, *Morinda citrifolia* L.

INTRODUCTION

The shift in the public interest to consume food that was free from chemical additive (particularly antibiotics), causes demand for organic broiler carcass (free from antibiotic residues) to be increased. Therefore, organic farm system was a promising future business (Yuniza dan Kusnadi, 2010). Organic farm system has not been widely applied by breeders in Indonesia. At the organic farm, the use of antibiotics should be avoided, so it needs to look for the replacement of the role of antibiotics and other additives that were safe for health. In this case, cinnamon leaf (*Cinnamomum burmanii*) and noni leaf and fruit (*Morinda citrifolia* L) could be an alternative to replace antibiotics, due to their rich and various phytochemical compound content. They were useful as antibacterial agent and could boost immunity. Yuniza and Kusnadi (2010) reported that the use of mixture of cinnamon leaves, noni leaves and field grass 8 to 12% in ration, besides can improve immunity but can also reduce abdominal fat, fat of thigh meat and thigh cholesterol. However, the use of cinnamon and noni leaf has not showed an increase in weight and even tended to decrease the weight of the chicken (although not significantly). This was due to the use of the forage causes crude fiber content of the ration to rise to chicken's limit to receive crude fiber. It was the existence of the high crude fiber that causes the limited amount of the forage (not more than 8% in ration).

The limited usage amount of cinnamon and noni leaf in ration causes the limited amount of phytochemical intake of the forage. Therefore, to make use the role of phytochemical of cinnamon and noni leaf to the fullest, extraction technique of the cinnamon and noni leaf mixture is needed, so that growth promoter agent, natural feed additive and feed supplement to replace the role of antibiotics can be obtained. These Cinnamon leaf

Corresponding Author: A. Yuniza, Faculty of Animal Husbandry, Andalas University, Padang, West Sumatra, Indonesia

and noni fruit and leaf mixture are called 'Cinnamononi Extract'. In Extracting the mixture of cinnamon leaf and noni fruit and leaf, the solvent and method used determine the yield and phytochemical compound in the resulting extract.

Based on the explanation above, this research needs to be conducted in order to find the appropriate extraction method or technique so that phytochemicals needed of cinnamon leaf and noni fruit and leaf mixture were not damaged and reduced in number. In addition, the research also conducted to determine the ability of cinnamononi extract generated, as a substitute for the role of antibiotics in inhibiting pathogenetic bacteria.

MATERIALS AND METHODS

There were two phases of experiment in this study, that were an experimental phase 1 to determine the phytochemical contents of 4 types of cinnamononi extract produced by four different extraction methods and an experimental phase 2 to determine the antibacterial activity of these four cinnamononi extracts against on *Escherichia coli* and *Salmonella* sp.

Materials: Materials required in the experimental phase 1 were: fresh cinnamon leaves, fresh noni leaves and fresh ripe noni fruit (yellow), methanol solvent and aquadest. Experimental phase 2 took cinnamononi extracts from Experimental phase 1 and bacteria stock of *Escherichia coli* and *Salmonella* sp, alcohol 70%, *Nutrient broth* (NB) powder, agar and tetracycline.

Research design: Experimental phase 1 was conducted to determine the phytochemical compound of these four cinnamononi extracts qualitatively. This qualitative test result will be expressed in negative statement or absent (-), weakly positive or low (+), positive or medium (++), strongly positive (+++) and very strongly positive or high (++++). Experimental Phase II was conducted to determine the antibacterial activity of the 4 types of cinnamononi extracts through completely randomized design (CRD), with five replications. There were 9 treatments of the combination of 4 types of cinnamononi extracts with 2 dose levels used. The treatments were as follow:

T = Tetracycline antibiotics with 0.002 g/ml dose, as a control
A1 = Cinnamononi extract type A, dilution concentration of 1 g/ml
A2 = Cinnamononi extract type A, dilution concentration of 0.1 g/ml
B1 = Cinnamononi extract type B, dilution concentration of 1 g/ml
B2 = Cinnamononi extract type B, dilution concentration of 0.1 g/ml
C1 = Cinnamononi extract type C, dilution concentration of 1 g/ml
C2 = Cinnamononi extract type C, dilution concentration of 0.1 g/ml
D1 = Cinnamononi extract type D, dilution concentration of 1 g/ml
D2 = Cinnamononi extract type D, dilution concentration of 0.1 g/ml

The measured variable was the clear zone formed on the agar medium used as bacteria inhibiting zone.

Making of cinnamononi extract: There were 4 types of extraction methods to obtain the cinnamononi extract, they are: method A, B, C and D. Methods A, B and C took dried cinnamon and noni leaf and dried noni fruits, while method D took fresh raw materials. The ratio of cinnamon leaves, noni leaves and noni fruit used was 1:2:1 based on dry material. The mixture of cinnamon leaves, noni leaves and noni fruit is referred as cinnamononi.

Type A stratified maceration extraction method by aquadest solvent: Dried cinnamon and noni leaves and noni fruits mixture of 50 g was dissolved in 250 ml aquadest (ratio 1:5), then it was let for 24 h in incubator shaker, it was filtered with filter paper (first extraction) afterward. The filtration residue was dissolved again by aquadest and second extraction was done. Filtrate obtained from the first and second extraction were combined and then it was dried in oven with 50°C temperature to form a paste which is referred as cinnamononi extract type A.

Type B stratified maceration extraction method by methanol solvent: Dried cinnamon and noni leaves and noni fruits mixture of 50 g was dissolved in 250 ml methanol (ratio 1:5), then it was let for 24 h in incubator shaker, afterward it was filtered with filter paper (first extraction). The filteration residue was re-dissolved by methanol and the second extraction was done. Filtrate obtained from the first and second extraction were combined and then it was evaporated to vaporize methanol with *rotary evaporator* at 50°C machine with speed of 80 rpm. The paste form is referred as cinnamononi extract type B.

Type C modification of distillation method: Fifty grams of cinnamon and noni leaves and noni fruits mixture was included in the distillation flask, then it was added aquadest as much as 250 ml (1:5). Distillation process was conducted later for 3-3.5 h to produce essential oil as an entrained component and phytochemical compound of dried filtrate in an oven with 50°C temperature. The formed paste was referred to as cinnamononi extract type C.

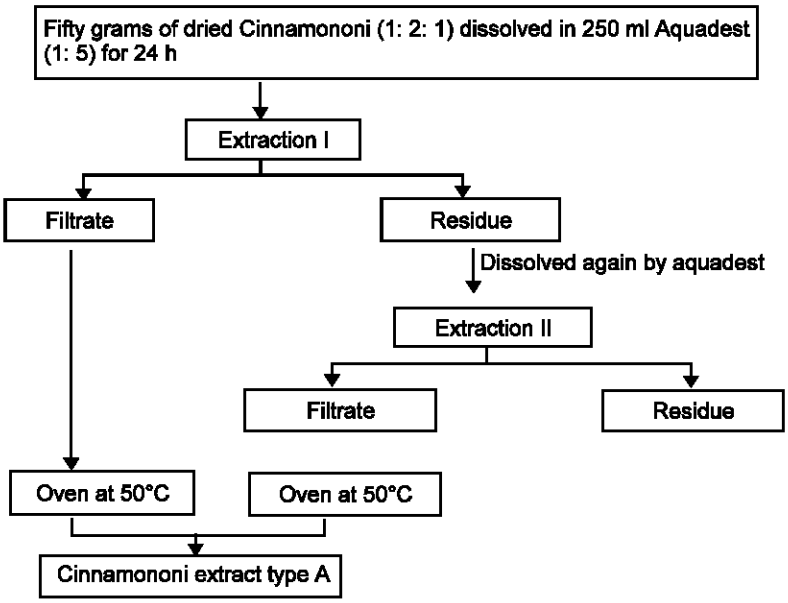

Fig. 1: Scheme of maceration technique with aquadest solvent (type A)

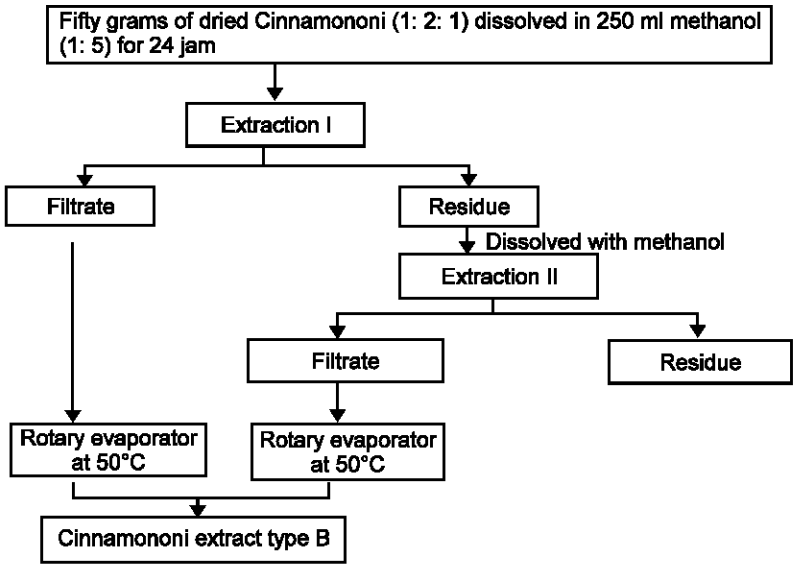

Fig. 2: Scheme of maceration technique with methanol solvent (type B)

Type D combination of maceration extraction method with distillation method: As much as 100 g of fresh cinnamon leaves were included in the distillation flask, by then aquadest of 400 ml (1:4) was added. The distillation process, thereafter, took 3-3.5 h to produce essential oil as an entrained component and liquid which contain several phytochemical compound as the extract of cinnamon leaves. The next step was the extraction of noni leaves and fruits in the following ways: 485 g of fresh noni leaves plus 432 g of fresh noni fruits with filtrate of fresh cinnamon from distillation process. They were macerated for 2 h, then were filtered. The filtrated residue was dissolved by using 100 ml of aquadest, they were macerated again and filtered again. The filtrate of the first and second filtration were combined and then was added an essential oil of the distillation of fresh cinnamon leaves. The filtrate was dried at 50°C to form paste which is referred to as cinnamononi extract type D.

Antibacterial activity test

Medium preparation for bacteria: Preparation of liquid medium was made of 8 g dissolved nutrient broth (NB) powder in 1 L aquadest, then it was heated and stir

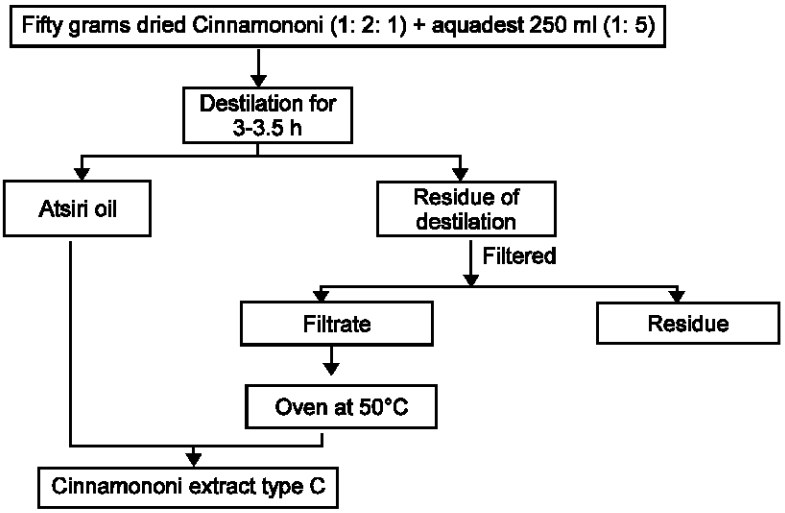

Fig. 3: Scheme of distillation technique (type C)

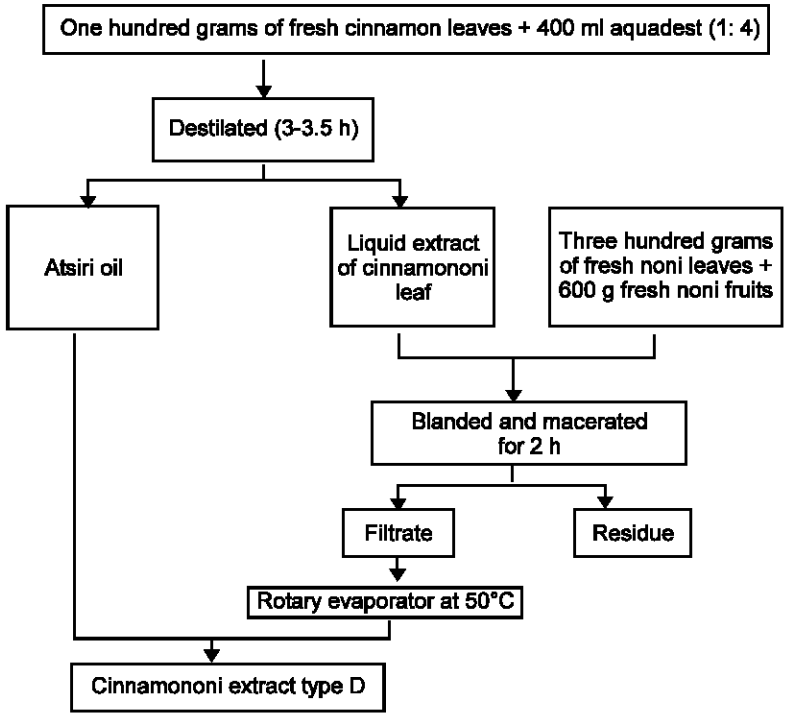

Fig. 4: Scheme of separated extraction technique (type D)

homogeneously. Solid medium was made of 8 g of NB powder and 20 g agar dissolved in 1 L aquadest, it was heated and stirred up homogeneously. All media were sterilized in autoclave at temperature of 121°C and pressure of 2 atm for 15 min.

Bacterial regeneration: Bacteria stocks (*Escherichia coli*, *Salmonella typhimurium*) on slant agar culture were taken in one loop and they were inoculated on erlenmeyer containing 10 ml of sterilized liquid medium. Erlenmeyer was incubated in incubator shaker at 37°C for 24 h.

Determination of antibacterial activity by using agar-ditch diffusion method: Determination of bacteria *E. coli* activity took 20 petri dishes containing 20 ml agar media and inoculum of *E. coli* with density of 0^6 CFU/ml. Petri dishes then shook up until medium and inoculum were

homogenous and let them to be condense. After they became solid, a well with perforator in diameter of 5 mm. Each well were filled with cinnamononi extract of the four types of 20 µl with 2 concentration levels: 1 g/ml and 0.1 g/ml. As a positive control tetracycline of 0.002 g/ml was used. Every treatment was repeated 5 times. It was, then, incubated at 37°C for 24 h and measured the inhibitory zone which indicates the antibacterial activity of *E. coli*. The same ways were done on *Salmonella* sp bacterial test, by adding inoculum bacteria of *Salmonella* sp with density of 10^6 CFU/ml on liquid medium.

Statistical analysis: Data were analyzed by analysis of Variance (ANOVA) using completely randomized design (CRD) as its experimental design (Steel and Torrie, 1991). Significant differences between treatment mean were tested using Duncan multiple rang test (DMRT).

RESULTS AND DISCUSSION

Analysis of phytochemical of cinnamononi extract: The use of different solvents and methods in extracting cinnamon and noni leaves mixture produce the following yields: extract A = 11.29%, extract B = 8.39%, extract C = 6.56% and extract D = 11.94%

Qualitative test result of these four cinnamononi extract showed that each of the extracts contain phenolic, alkaloids, saponins and tripernoids compounds, but with different levels, meanwhile the levels of flavonoids only exist in cinnamononi extract type A, C, and D (Table 1).

Flavonoid compound was not found in extract B. It indicated that flavonoid compound in cinnamon and noni leaf and fruit were flavone glycosides which tend to dissolve in polar solvent. The solvent used to obtain cinnamononi extract type B was methanol with polarity lower than water, so that the flavone glycosides could be seen in extract A, C and D which used aquadest as solvent and had higher polarity than that of water. Flavonoid compound was, generally, easy to dissolve in water, particularly in the form of its glycosides.

Antimicrobial potential in Cinnamononi extract type A, C and D was determined by the combination of strong phenol content, medium alkaloids and the present of saponins and triterpenoid. Meanwhile in type B, it was prominently determined by the strong triterpenoid and was supported by medium phenolic, less saponins and alkaloids contents.

Antibacterial activities of cinnamononi extract on *E. coli* and *Salmonella* sp: Antibacterial activities are determined with the measurement of inhibition diameter of zone on growth of *E. coli* and *Salmonella* sp. The antibacterial activities from four types of cinnamononi extracts were presented in Table 2.

Table 1: Phytochemical compound of four types of cinnamononi extract

	---------- Types of cinnamononi extract ----------			
Compound	Type A	Type B	Type C	Type D
Phenolic	+++	++	+++	+++
Saponins	+	+	+	++
Alkaloids	++	+	+	++
Triterpenoids	+	+++	+	+
Flavonoids	++	-	++	++

(-) = negative or absent
(+) = weakly positive or low
(++) = positive or medium
(+++) = strongly positive or strong enough

Table 2: Antibacterial activities of cinnamononi extract on *E. coli* and *Salmonella* sp

	----- Mean ID of zone (mm) -----	
Treatments	*E. coli*	*Salmonella* sp
T = Tetracycline, 0.002 g/ml	21.2^{bc}	9.6^{e}
A1 = Type A 1 g/ml	24.6^{a}	24^{a}
A2 = Type A 0.1 g/ml	19.2^{d}	8.8^{e}
B1 = Type B 1 g/ml	21.4^{bc}	21.2^{bc}
B2 = Type B 0.1 g/ml	10.4^{d}	9.8^{e}
C1 = Type C 1 g/ml	23.8^{ab}	23.4^{ab}
C2 = Type C 0.1 g/ml	13^{d}	13.4^{d}
D1 = Type D 1 g/ml	21.2^{c}	19.2^{c}
D2 = Type D 0.1 g/ml	11.2^{d}	11.2^{de}

ID: Inhibition diameter

The result of statistical analysis indicated that treatments were significant ($p<0.01$) to inhibition diameter of zone on *E. coli* and *Salmonella* sp. After DMRT test conducted, it could be seen that treatment A1, showed significantly greater zone of inhibition on *E. coli* ($p<0.05$) than tetracycline, whereas treatments B1, C1 and D1 showed non-significant zones of inhibition ($p>0.05$) with tetracycline on *E. coli*. The data also showed that treatments C1 was non-significant to zone diameter of inhibition of A1. The DMRT test also showed that treatments A2, B2, C2 and D2 with a lower dose (dilution of 0.1 g/ml) made diameter of clear zones was significantly lower than tetracycline ($p<0.05$).

The data indicated that by using dose 1 g/ml, the four types of cinnamononi extracts have been able to match and even exceeded (i.e., A1) tetracycline ability in inhibiting the growth of *E. coli*, but their ability became lower of tetracycline ability if cinnamononi extract dose is reduced to 0.1 g/ml.

Against bacterial growth of *Salmonella* sp, DMRT result showed that treatments A1, B1, C1, D1 and C2 produced zone of inhibition significantly greater than tetracycline ($p<0.05$), whereas no significant differences in between treatments A2, B2 and D2 with tetracycline ($p>0.05$) to zone diameter of inhibition. The data also showed that there was no treatment which produced zone diameter of inhibition lower than tetracycline. DMRT result showed that cinnamononi extract given with a lower dose, that is 0.1 g/ml, were able to match and even exceeded (i.e., C2) tetracycline ability in inhibiting the growth of *Salmonella* sp.

This bacterial activity could be attributable to the phytochemicals in the extract (Enemuor *et al*., 2011). Antibacterial activity of three types cinnamononi extract (i.e., A, C and D) was due to its high content of phenolic and also was supported by the present of alkaloids, saponins and flavonoids; whereas antibacterial potential from type B of cinnamononi extract was given by the high content of triterpenoid. The role of phenol as an antibacterial was by denaturing bacterial protein through absorption involving hydrogen bonds. At the high level, phenol causes coagulated protein and cell membrane undergo lysis, thereby change the permeability of the bacterial cell membrane (Siswandono and Soekardjo, 2000). Antibacterial properties of phenol were also investigated by Pambayun (2007) which stated that the phenolic compound in the gambier extract play role in inhibiting the growth of *S. aureus*.

High level of tripernoid in cinnamononi extract type B gave antibacterial potential on the extract. Terpenoids can be an antibacterial by damaging bacterial cell membrane (Cowan, 1999). Two kinds of terpenoid (*Phytadiene* and 1,2-*seco-cladiellan*) containing in stone breaker herb (*Phyllanthus niruri* Linn) proved to be active against *S. aureus* and *E. coli* (Gunawan *et al*., 2008). Kaurenoic acid terpenoids of Pseudognaphalium vira vira can damage cell membranes of *S. aureus* through hydrogen bonds kaurenoic acid carboxylic group with phosphoryl oxygen atom of cell membrane i.e., CO_2H-O = P with a distance of 1.91A (Urzua *et al*., 2008).

Conclusion: Based on the data, it can be inferred that cinnamononi extract type A, B, C and D has the ability to inhibit the growth of *E. coli* dan *Salmonella* sp. Cinnamononi extracts with the most excellent antibacterial potential to inhibit *E. coli* were type A (macerated and stratified with water solvent) and type C (modification of reflux extraction method), but the most excellent antibacterial potential to inhibit *Salmonella* sp. was type C.

REFERENCES

Cowan, M.M., 1999. Plant product as antimicrobial agents. Clinical Microbiology Reviews, 12: 564-582.

Enemuor, S. Chukwuemeka, Omale, James, Joseph and E. Matthew, 2011. Antibacterial activity: a comparison of ripe and unripe fruit extract of *Cissus multistriata (vitaceae)* plant. J. Bacteriol. Res., 3: 83-87.

Gunawan, I.W.G., I.G.A.G. Bawa and N.L. Sutrisnayanti, 2008. Isolation and Identification of Active Antibacterial Terpenoid Compound in Stonebreaker Herb (*Phyllanthus niruri Linn*), Chemistry Department, Udayana University, Bukit Jimbaran, Chem. J., 2: 31-39.

Pambayun, R., M. Gardjito, S. Sudarmadji and K.R. Kuswanto, 2007. The Content of Phenol and Antibacterial Nature of Various Species of Gambier Product (Uncaria gambir Roxb.), Majalah Farmasi Indonesia, 18: 141-146.

Siswandono and Soekardjo, 2000. Kimia Medisinal 2, Airlangga University Press, Surabaya, 99, 112, 116, 151.

Steel, R.G.D. and J.H. Torrie, 1991. Principles And Prosedures Of Statistics . 2nd Edition. Translated by Sumantri, B., PT. Gramedia Pustaka Utama, Jakarta.

Urzua, A., M.C. Rezende, C. Mascayano and L. Vasquez, 2008. A Structure-Activity Study of Antibacterial Diterpenoids, Molecules, 13: 882-891.

Yuniza, A. and E. Kusnadi, 2010. Study of the Use of Earthworm and Various Fresh Forage in Broiler Rations towards Organic Farm. Research Report (unpublished).