Potency of Persimmon Fruit (*Dyospiros kaki*) As an Organic Antibiotic, Antifungal and Anthelmintic on the Livestock: an Analysis

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ABSTRACT

Indonesia has an abundant of plants which are potential to be used as natural medicine. An effort must be made to help maintain those potential medicinal plants in Indonesia. One method is to carry out researches on Indonesian medicinal plants to support the health of livestock so that the nutritional needs of the community are fulfilled.

Previous Researches reported that Persimmon fruit (*Dyospiros kaki*) has a strong potential as a substitute for antibiotics, antifungal and anthelmintic use in livestock. This research was conducted to analyze the microbiological content of Persimmon fruit after going through the process of auto fermentation. Auto fermentation was carried out for 2 weeks in an anaerobic stainless tank. Microbiological identification was done at Bogor Veterinary Research Center. Identification of worm's eggs was carried out using Mc. Master Method.

The results showed that *Dyospira kaki* fermentation was rich in *Bacillus sp* as much as 1.05 x 10^3 cfu / ml which is useful as an antibiotic and organic antifungal. The content of pathogenic bacteria *E coli* and *Staph aureus* were negative. The research on the use of herbs to reduce worms was done with 20% *Curcuma mangga*, 20% C*urcuma domestica*, 20% *Curcuma xanthorrhica*, 40% *Dyospiros kaki* fermented, This research showed that cows supplemented with 60 ml OSE from laboratory test, decreased the number of worm eggs. The percentage of decrease was 73%, from 220 worm's eggs to 60 eggs.

Keywords: *Dyospiros kaki*, autofermentation, *Bacillus sp*, antibiotic, antifungal, anthelmintic

INTRODUCTION

We deserve to be grateful to be born and living in Indonesia, a vast country, rich with so many precious things on earth and it's fertile and prosperous soil and always warm temperatures. But we are concerned about the medicinal plants that once encountered now have begun to disappear, so it must be imported.

Tjandra Yoga Aditama (2015) stated that 4 scope of research of Medicinal Herbs and Medicinal Plants that have been done at Health Research and Development Agency of Ministry of Health is research of sanctification of herbal medicine, research of medicinal plants, laboratory-based development and research of medicinal plants and herbs (Ristoja).
In Ristoja 2012 year successfully inventoried 15,773 herbs from 209 ethnic groups and successfully identified 1,740 species of medicinal plants from 13,576 names of medicinal plants (60% of total data). The number of herbariums collected was 13,398 herbariums. From the inventory results then in 2014 carried further analysis.

Jamu in addition to human health can also be used for livestock health. Livestock is a commodity source of animal protein that is needed by the community, so to obtain optimal growth, livestock health is very important to note. It is therefore necessary to exploit Indonesia's abundant natural wealth of natural medicine as a substitute for the use of chemical antibiotics, antifungal and anthelmintic in livestock to help realize healthy Indonesian society.

MATERIALS AND METHODS

General

Auto fermentation method.

1. Persimmon fruit washed and stems discarded.
2. So be sliced and input to a sterile container and closed tightly, air tight not expose to direct sun light as long as 2 weeks.
3. Processes anaerobe auto fermentation will happen.
4. Then take the liquid of Persimmon fermented.

Isolation bacteria method.

1. A total of 0.1 ml samples were poured aseptically on LB agar and incubated for 24 to 48 hours at 37 ° C.
2. The growing bacterial colonies were morphologically observed and then the hemolysis colony was randomly drawn with ose and in culture on LB medium repeated until obtained Colonies of pure *Bacillus sp* bacteria.
3. Also included bacteria *Bacillus sp* standard ATCC 6633 as a positive control.

Bacterial Identification:

1. Gram staining.
    a. Gram stain uses a newly grown Bacillus culture (bacteria incubated for 16 hours at 37 ° C).
    b. The standard staining procedure uses three staining processes i.e. violet crystals, decolorization and counterstaining with safranin.
2. Motility Test.
    a. To detect Bacillus motility using the hanging drop technique.
    b. One drop (drop). The bacterial suspension is placed on a cover slip glass coated with oil.
    c. Then the object glass is placed on the slip cover with slowly launched then the object glass is reversed and inspected using a 40x optical microscope to see the bacterial motility.

Biochemistry Test:

1. Test catalase.
    a. Newly grown bacterial cultures (incubated for 16 ± 2 hours) are smeared on top of the glass.
    b. The slide is clean, then drops 3% H_2O_2 and allowed to react for 30 seconds.
    c. There is observed and no reference to determine positive and negative catalase.
2. Hydrolysis test carbohydrates.
   Newly planted bacterial cultures were inoculated into a blood-agar-containing blood agar plate that had been added 1% starch and incubated at 37ºC for 24-48 hours.
    a. After incubation, The cup was flooded with a few drops of iodine and observed the presence of clear zones around the colony.

   b. The presence of clear zones around the Colony shows positive hydrolysis of starch.
3. Test hemolysis.
   a. The hemolytic test of *Bacillus sp* bacteria isolated culture was verified using blood agar.
   b. Newly grown bacteria are inserted in the agar blood and incubated at 37 ° C for 24 hours.
   c. The hemolytic reaction is obeyed by the presence of a hydrolysis zone around the colony (hemolysis).
4. Citrate Test.
   a. Aseptic bacterial cultures were scratched on to simile citrate and incubated for 48 hours.
   b. Positive results are indicated by the growth of bacteria on the tilting agar and at the same time there is a change of media color from green to blue (pH indicator).
5. Test Voges-Proskauer (VP).
   a. Bacillus isolates were inoculated in tubes containing broth glucose phosphate and incubated for 48 hours.
   b. After incubation, the Barritt reagent consisting of a mixture of alcoholic α-naphthol and 40%.
   c. Potassium hydroxide solution is added to the tube and shaken.
   d. The tube was left standing for 15 minutes.
   e. The presence of red discoloration within 15 minutes after the addition of Barritt reagent shows the presence of acetoin and as a positive reaction.

Physiologic Test

1. Growth test at various pH.
   a. LB broth is made aliquot with different pH (2.0, 4.0, 6.0, 8.0 and 10.0) by adding 0.1 N NaOH / HCl then in Sterilization with autoclaving.
   b. Aliquot of sterile LB broth (5 ml) was inoculated with newly-growing bacteria of 50 µl (105-106 CFU / ml).
   c. The aliquots were incubated at 37ºC for 24 to 48 hours and observed growth characterized by turbidity change.
2. Test growth at various temperatures.
   a. Aliquot LB broth (5 ml) was inoculated with 50 µl of newly-growing bacteria (105-106 CFU / ml) and incubated at different temperatures (4, 15, 25, 35, 45 and 55 ° C) for 24-48 hours.
   b. After incubation the tube was observed for growth characterized by turbidity change.
3. Growth test on various salt concentrations.
   a. The LB broth tube was prepared with different salt (NaCl) concentrations (4%, 6.5% and 8%) then inoculated with 50 µl of newly grown bacteria (105-106 CFU / ml) grown and incubated at 37 ° C for 24 to 48 hours.
   b. After incubation, bacterial growth was observed based on turbidity change.

Identification of worm egg used Mc. Master Method.

1. Three grams of ground samples take using mortal
2. Enter a saturated 60 ml saturated solution and stir until homogeneous
3. Filtered and put in a glass baker
4. Enter with the pipette to the count room (whitlock) until fully charged

5. Check under a microscope with magnification 4 x 10
6. Match the worm eggs with the picture.

RESULTS AND DISCUSSION

Dyospiros kaki fermented in this research contained bacteria in Table 1.

Table 1. Isolation and identification microbiology of persimmon fermentation

Microbiologis	Replication 1	Replication 2
Bacillus. Sp	1,0 x 10^3 cfu/ml	1,1 x 10^3 cfu/ml
E coli	Negative	Negative
Staph. Aureus	Negative	Negative

Bacillus.sp is a gram-positive bacterium with stem cells measuring 0.3-2.2 x 1.27-7 micro meters, some motile, if in heat will form endospores, aerobic to facultative anaerobes, metabolism with fermentation and respiration. Several studies have successfully isolated and purified *Bacillus sp*. Positive Gram is subtilin produced by *Bacillus subtilis* (Klein et.al., 1993), megacin produced by *B. megaterium* (Tagg et al., 1976), coagulin produced by *B. coagulans* (Hyronimus, 1998), cerein is produced by *B. cereus*, and tochicin produced by *B. thuringiensis* (Paik et al., 1997).

The antibiotic compounds produced by *Bacillus. sp* is bacitracine, pumulin, laterosporin, gramisidin, and tirocidin which are effective against Gram positive bacteria and colistin and polymyxin are effective against Gram negative bacteria. While difficidin have a wide spectrum, mikobacilin and zwittermicin are antifungal (Todar, 2005).

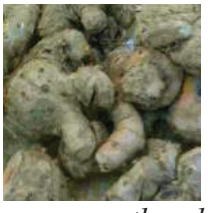
Bacillus sp has the ability to produce antibiotics that play a role in nitrification and denitrification, nitrogen binders, selenium oxidizers (Se), manganese oxidizers and reducers (Mn), dissolved carbonate, can dissolve phosphate, and decrease pH Substrate due to the resulting organic acid, can mineralize the organic material complex either in the form of polysaccharide compounds, proteins and cellulose

Bacteriocins have been proposed as a replacement for antibiotics to which pathogenic bacteria have become resistant. Potentially, the bacteriocins could be produced by bacteria intentionally introduced into the patient to combat infection. In the last years, several studies on bacteriocins have demonstrated that the optimization of their production conditions, their purification methods, their combinations with other antimicrobial agents, the hurdle technology approach, and nanotechnology formulations, could all represent solutions to some of the previously mentioned problems.

The research on the use of herbs to reduce worms has done with the composition of 20% *Curcuma mangga*, 20% C*urcuma domestica*, 20% *Curcuma xanthorrhica*, 40% *Dyospiros kaki* fermented, named Organic Supplement Energizer (OSE). This composition contained astir oil, tannins, amylin, fructose, flavonoid, vitamin C, Fe, Cu and Phosphor. These functions are anti bacteria, antidote, analgesia, antipyretic, and decrease stomach acid.

Curcuma mangga

Curcuma domestica



Curcuma xanthorrhiza

Figure 1. Composition of Organic Supplement Energyzer (OSE)

This Research indicated that cow supplemented with OSE 60 ml from laboratory test, a number of worm eggs decrease. Identification of worm egg used Mc. Master Method sees in Table 2.

Table 2. Identification Eggs of Endoparacyte on Cow before and after giving OSE

Endoparasit	Before OSE (EPG)	After OSE (EPG)
Nematoda		
Strongyle sp	150	60
Strongyloides sp	27	0
Protozoa		
Coccidia sp	+	0
Moniezia sp	40	0
Trematoda		
Fasciola sp	2	0
Paramphistomum sp	1	0
TOTAL	220	60

OSE effective to decrease eggs of worm and protozoa. Recommendation this research give OSE to cow will be increased cow production.

CONCLUSIONS

1. Fermentation *Dyospiros kaki* contains *Bacillus sp* bacteria as much as 1.05 x 103 cfu / ml which is useful as an antibiotic, antifungal and anthelmintic organic.
2. The content of pathogenic bacteria *E coli* and *Staph aureus* negative.
3. OSE can decrease eggs worms from 220 EPG to 60 EPG in cow.

REFERENCES

Hyronimus B, Le Marrec C, and Urdacim MC. (1998) Coagulin, a bacteriocin-like inhibitory substance produced by *Bacillus coagulans* I4. *J Appl Microbiol* **85**, pp 42–50.

Klein C, Kaletta C, Schnell N, and Entian KD. (1992) Analysis of genes involved in biosynthesis of the lantibiotic subtilin. *Appl Environ Microbiol* **58**, pp 132–42.

Paik HD, Bae SS, Park SH, and Pan JG. (1997) Identification and partial characterization of tochicin, a bacteriocin produced by *Bacillus thuringiensis* subsp. *tochigiensis*. *J Ind Microbiol Biotechnol* **19**, pp 294–8.

Retno Widyani dan Muh. Hisyam Hermawan. Nutritional Value of Persimmon Yoghurt (*Diospyros kaki*) as Healthy Soft Drink to Make Healthy and Fittness: An Analysis. Proceeding The First International Conference Technology on Biosciences and Social Sciences. Industry based on Knowledge. Andalas University. Padang.

Retno Widyani, Moch. Hisyam Hermawan, Fitri Dian Perwitasari, Ida Herawati. 2016. Efektifiktas Organik Suplemen (OSE) Terhadap Helminthiasis Pada Sapi Potong. Proseding Seminar Nasional Pengembangan Sumberdaya Ternak Lokal Dalam Pencapaian Sustainable Development Goals. Fakultas Peternakan. Universitas Padjadjaran Bandung.

Tagg J.R., A.S. Dajani and L.W.Wannamaker. 1976. Bacteriocins of gram positive bacteria. Bact. Rev., 40: pp 722-56.

Tjandra Yoga Aditama. 2015. Catatan Kegiatan 2015. Penelitian, Terapannya & Kesehatan Masyarakat.Lembaga Penerbit Balitbangkes. Jakarta.

Todar Kenneth. 2005. Todar's Online Texbook of Bacteriology. http://www.textbookofbacteriology.net/Bacillus.html.