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Ovicidal Effect of Ground Mature Papaya Seeds (*Carica papaya* L) on Eggs of Round Worm (*Ascaris suum*)

Peran Ovicidal Herbal Serbuk Biji Pepaya (*Carica papaya L*) Matang Terhadap Telur Cacing *Ascaris suum*

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Abstract

An *in vitro* experiment to determine the effect of ground mature papaya seeds on characteristics of *Ascaris suum* eggs was conducted employing a Randomized Block Design. Four doses of the ground preparation – 0 mg (as control), 285 mg, 570 mg, and 855 mg- in 40 ml of physiological saline solution – were used to soak 100 eggs. Six replications were made. Worm eggs were obtained from the uteruses of ascariasis pigs slaughtered at the local abattoir. The eggs characteristics observed after application of the treatment were the embryo formation ability and the damage of egg cell layers. Statistical analysis was applied to the data collected using Analysis of Variance followed by Least Significance Test. The results showed that the ability of treated eggs to form embryos was significantly lower than that of control. Such a decrease in embryo formation ability could be related to the formation of granule (albumen coagulation) in the egg cell layers after the treatments. All treated eggs possessed such granules.

Key words: Herbal treatment, ground mature papaya seeds, parasite, swine, *Ascaris suum*

Abstrak

Penelitian peran ovisidal herbal serbuk biji pepaya matang terhadap telur cacing *A. suum* (penurunan daya berembrio dan kerusakan kulit telur cacing *A. suum*) menggunakan Rancangan Acak Lengkap yang terdiri dari 4 perbedaan dosis herbal serbuk bici pepaya matang untuk merendam 100 butir telur cacing *A.suum* selama 24 jam (0 mg/40 ml NaCl fisiologis, 285 mg/40 ml NaCl fisiologis, 570 mg/40 ml NaCl fisiologis dan 855 mg/40 ml NaCl fisiologis) dan 6 ulangan. Penelitian ini dilakukan secara *in-vitro* menggunakan telur cacing *A. suum* yang diperoleh dari uterus cacing *A. suum* dari babi penderita ascariasis, yang dipotong di RPH Sanggaran, Denpasar. Data perbedaan daya berembrio dianalisis dengan menggunakan uji Sidik Ragam dengan uji F pada taraf nyata 5%, bila terdapat perbedaan bermakna dilanjutkan dengan uji Beda Nyata Terkecil. Untuk mengetahui berbedaan kerusakan kulit telur cacing dilakukan analisis deskriptif. Hasil analisis menunjukkan bahwa daya berembrio telur cacing *A. suum* yang mendapat perlakuan lebih rendah daripada kontrol, secara *in-vitro*. Penurunan daya berembrio ini mungkin ada hubungannya dengan granulasi (koagulasi albumin) kulit telur cacing *A. suum* yang mendapat perlakuan. Telur cacing *A. suum* yang mendapat perlakuan semuanya mengalami granulasi (koagulasi albumin) pada kulitnya.

Kata kunci: Pengobatan serbuk biji pepaya matang (HSBPM), Parasit, Babi, *Ascaris suum*

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Introduction

Ascariasis, caused by infection with the worm *Ascaris suum*, a parasitic and zoonotic disease of pigs that still can not be controlled thoroughly and causes severe economic loss (Nejsum *et al*., 2005; Roepstorff, 1998). The prevalence is high. For example, in Denmark it ranges between 25−35% (Roepstorff *et al*., 1998) and in Bali it was reported at around 34.45% with an average 387.5 eggs per gram feces (Suweta, 1994). The difficulty in controlling *A. suum* infection is due to the ability of the eggs to survive in the external

environment (Stevenson, 1979); the egg cell layer is thick and with an inner lipid layer that protects them from chemical and thermal damages.

It is well known that some worm drugs, i.e. albendazole can be used to effectively destroy the egg cell layers and thus kill the eggs. However, such medication is still considered expensive for traditional pig farmers such as those in Bali. The animal can easily become resistant after prolonged application. Moreover, its residue in pork may cause a harmful effect on customers. Therefore, attempts should be made to find alternative medication particularly of herbal origin that can damage the eggs,is cheap in price, and easily available.

Some research has been published on the use of herbs such as pineapple, young papaya seeds, and papaya latex as worm medication. Suweta *et al*., (1991) reported that application of papaya latex at 5% and 15% to *A. suum* eggs *in vitro* significantly reduced the number of eggs that grew into embryos. Similarly, Satrija *et al*., (1994) found that papaya latex at 8 g/kg body weight applied to pigs killed up to 100% of *A. suum* eggs at Day 7 after treatment; however, the mechanism of action was not clear. Later, Suweta (1995) reported that papaya latex has an ovicidal effect in that it can kill *A. suum* eggs. An *in vitro* ovicidal effect of ground young papaya seeds was observed in terms of reduced embryo formation in *A. suum* and also in *A. lumbricoides*. Examination of unfertile *A. lumbricoides* eggs under an electron microscope revealed that the egg cell layer become thinner or showed material coagulation in the external part (Bariah *et al*., 2001). In an *in vivo* study, Suweta (1996) reported that a ground preparation of young papaya seeds at 0.3 g/kg of body weight can effectively prevent the development of eggs into mature worms.

Singh and Nagaich (1999) reported, from their *in vitro* study, that papaya seed extract can significantly reduce glucose uptake by *A. galli* and reduce the glycogen contents. Other *in vitro* works have found that 100-300 µmol of benzyl-isothiocyanate found in papaya seeds can inhibit energy metabolism and activity of motor nerve in *A. galli*.

As the use of mature papaya seeds in combating worm infection, particularly of *A. suum*, has not been reported, the present study was conducted to determine whether such a herbal preparation can be used effectively to control thise worm infection. Thus, the current work was aimed to study the effect of mature papaya seeds on the ability of *A. suum* eggs to develop into embryos and their ability to destroy the egg cell layers. Mature seeds can be found easily and are considered as waste,so, it is worthwhile to assess their potential for utilization in this way.

Materials and Methods

The *in vitro* study was conducted following a Completely Randomized Design with 4 treatments and 6 replications as shown in Table 1 (Zainuddin, 1999). Eggs were collected from uterus of pigs suffering from *A. suum* infection (ascariasis) slaughtered at the local abattoir. Preparation of herbal ground of mature papaya seeds followed that of reported by Suweta (1994).

Experimental works were carried out at the laboratory of Center of Study on Animal Diseases, Faculty of Veterinary Science, Udayana University, Bali and in Electron Microscopy Unit of Airlangga University, Surabaya.

Assessments on embryo formation ability of eggs after the treatments were as follows. Samples of egg (8 eggs/µl diluter) were soaked in prepared solutions as above at room temperature. Every day for a period of 30 days the eggs were aerated and checked for embryo formation. A total of 100 eggs per replication were examined for the percentage of eggs growing into embryo. Statistical analysis using Analysis of Variance followed by Least Significance Test was applied to the data collected (Hadi, 1976; Gomez, dan Gomez, 1995; Steel and Torrie, 1980)

Evaluation on the effect of the treatment on external egg cell layer was performed as followed. At Day 11, the eggs were fixated in 4% formaldehyde and then examined under scanning electron microscope (SEM) for possible damages as reported by Bariah *et al*., (2001).

Results and Discussion

Effect on Embryo Formation

The effects of treatment with different doses of ground mature papaya seeds on embryo formation at early stage of embryo development (15 days) at the end of development (21 days), and at 30 days post-incubation *in vitro* were shown in Table 2.

From Table 2, it can be clearly observed that the control eggs with no herbal treatment – had very high rates of embryo formation for all 3 times of examination (i.e at 15 days, 21 days, and 30 days after incubation). In contrast, the treated eggs had significantly lower ($P < 0.01$) rate of embryo formation. The rates of embryo formation for the 3 treated groups showed no significant difference.

The present results on rate of embryo formation in physiological saline solution (the controls eggs), which was still high even 30 days after incubation are in accordance with those reported by (Stephenson *et al*., 1977). They found that the rate of embryo formation *in vitro* for *A. suum* eggs at 30 days post-incubation was more than 95%. In the current study it was found that the rates of embryo formation for eggs subjected to treatment with herbal preparations (the 3 treated groups) were low, so it can be concluded that mature papaya seeds prepared as ground herbal preparation has an ovicidal effect on *A. suum* eggs; thus, it can be effectively used to control worm infection.

Previous studies have reported that the vermicidal and ovicidal effects of young papaya seeds are related to their content of active substances such as papain enzyme (0.015%), glycosides (2.81%), and other unidentified active substances (Suweta, 1996). Moreover, Duke (2004) reported that the active substances found in papaya seeds that can work as medication for controlling worm (anthelmintic) are alkaloids (carpain and carpasemine) and glycosides (benzyl-isothiocyanate). It has also been reported by other workers that the alkaloid carpain present in papaya seeds can kill *Ascaris* spp. (Duke, 2004; Suweta, 1996 and Yongabi, 2005) and benzyl isothiocyanate is known to have strong anthelmintic properties (Bariah *et al*., 2000). However, the active substances in mature seeds have not been reported to date.In addition, the effective dose of such herbal preparation for combating worm infections has not previously been reported. Therefore, their application at the level of pig farming still need to be elaborated.

Table 1. Treatments, replications and number of eggs assessed for each treatment.

Treatments	Replications and Number of Eggs	
0 mg /40 ml NaCl (P0)	6 x	100
285 mg/40 ml NaCl (P1)	6 x	100
570 mg/40 ml NaCl (P2)	6 x	100
855 mg/40 ml NaCl (P3)	6 x	100

Note:

1. P0: Control group: eggs soaking in 40 ml of physiological saline solution (physiological NaCl) for 30 day
P1: eggs soaking in 285 mg of herbal preparation in 40 ml of physiological NaCl for 24 hours, followed by rinsing and incubating in physiological NaCl for 30 days. The dose of 285 mg of herbal preparation is equal to 0.3 g/kg of body weight.

Table 2. Average rate of embryo formation of *A. suum* eggs after soaked in different doses of mature papaya seeds for 24 hours examined at Day 15, Day 21, and Day 30 post-incubation.

Dose of Herbal Preparation	Percentage of Embryo Formation			*p*
	Day 15	Day 21	Day 30	
0 mg/40 ml	58.67 ± 5.502	96.333±1.966	97.833±1.169	0.001*
285 mg/40 ml	2.86±2.229	3.500±2.429	3.500±2.429	0.587
578 mg/40 ml	1.833±1.941	2.667±2.503	2.167±2.401	0.651
855 mg/40 ml	2.000±0.3	2.667±2.503	2.667±2.503	0.928

*significant at $p < 0.05$

Effects on The Egg Cell Layer

Scanning electron microscopy (SEM) study demonstrated that by Day 11 after treatment, *A. suum* eggs showed the presence of granules in their cell layers (Table3). Formation of the granules was as a result of coagulation of albumen.

All (100%) eggs from the control group showed no granule formation by Day 11 after incubation. In contrast, all eggs from the 3 groups treated with ground mature papaya seeds (285, 578 and 855 mg/40 ml) did not exhibit such granules. From SEM photographs taken (Figures 1-4), it can be seen that the egg cell layers thickened in certain parts and became thinner in other parts. This may indicate that changes in the egg cell layers occurred without any involvement of materials from the inner part of the eggs.

Thus, from examination under SEM it was obvious that all eggs subjected to treatment with the 3 doses of ground mature papaya seeds experienced damages to their cell layers. Parts of the layer thickened, but it became thinner in the other parts, which may indicate pathologic changes in the albumen layer. It is well known that papain enzyme in papaya has a proteolytic activity. Moreover, papaya seeds also contain alkaloids (carpain and carpasemine) and glycosides (benzyl isothiocyanate) that may have effect on the morphology of the egg cell layer.

Formation of granules observed in *A. suum* eggs in the current study may indicate that enzymes and other substances from mature papaya seeds may have effects on the egg cell layer in the form of coagulation of the albumen layer, and that, in turn, may destroy the ability of the eggs to develop into embryos. The present results seem in accordance with the previous findings of Bariah *et al*., (2001) whose reported that coagulation of the egg cell layer inhibited formation of embryos in *A. lumbricoides*. This is probably due to proteolytic effect of papain content on papaya to albumen on egg worm *A. suum* shell.

In conclusion, application of ground mature papaya seeds to *A. suum* eggs resulted in coagulation of the albumen layer. Consequently, the eggs lacked the ability to grow into embryos; the rate of embryo formation was severely reduced. Since,it is still not known what are the active substances in mature papaya seeds that arecresponsible for the observed changes in the morphology of the treated eggs is still not known, further study is suggested.

Table 3. Examination of *A suum* eggs under SEM for the presence of granules in egg cell layers.

Treatments	Granulation (Albumen coagulation)			Total	Percentage
	Egg 1	Egg 2	Egg 3		
0 mg/40 ml	0	0	0	0	0 %
285 mg/ 40 ml	1	1	1	3	100 %
578 mg/ 40 ml	1	1	1	3	100 %
855 mg / 40 ml	1	1	1	3	100 %

Note
0: no granules observed in egg cell layer (normal)
1: granules were observed in egg cell layer

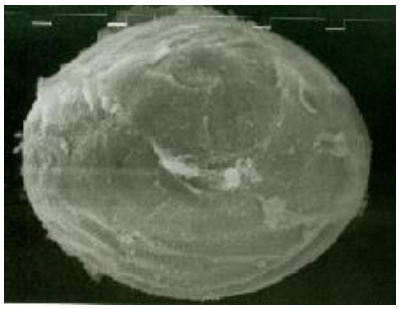

Figure 1. Morphological appearance of the egg cell layer of the P0 (control) egg that lookeds normal at Day 11 after incubation. Scale 2000:1

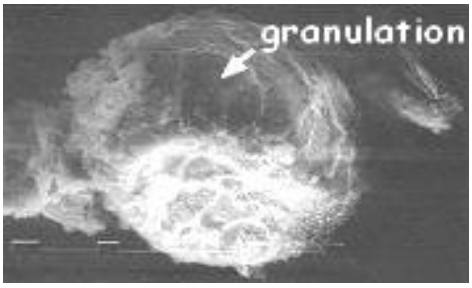

Figure 2. Morphological appearance of the egg cell layer of P1 eggs (treated with ground mature papaya seed (285 mg/40 ml) at Day 11 after the treatment; granulation can be observed. Scale 1:5000

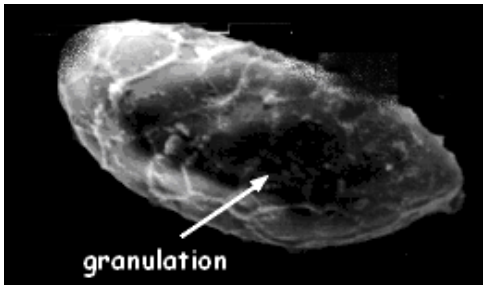

Figure 3. Morphological appearance of the cell layer of *A. suum* eggs at Day 11 after herbal treatment at a dose of 578 mg/40 ml. Granulation can be observed. Scale 1:8000

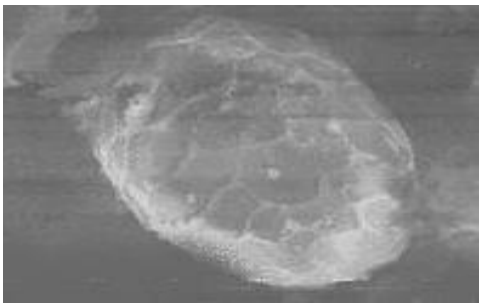

Figure 4. Morphological appearance of the cell layer of *A. suum* eggs at Day 11 after herbal treatment with 855 mg/40 ml. Scale 1:11000

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