FEASIBILITY OF USING PAPAYA SKIN EXTRACT FOR DIGESTION OF SWAMP EEL VISCERA FOR HARVESTING INFECTIVE STAGE LARVA OF *GNATHOSTOMA* SPP

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Abstract. In this study, we reported the feasibility of using papaya skin extract (*Carica papaya* L.) as an alternative to enzyme pepsin in harvesting *Gnathostoma spinigerum* third-stage larvae. From experimental digestion, we found that the different numbers of recovered larvae between papaya skin extract and pepsin were not statistically significant ($p > 0.05$). When the derived larvae from pepsin and papaya skin extract digestion were cultivated in BME medium for 7 days, the survival rates were not significantly different either ($p > 0.05$). Thus, papaya skin extract might be another choice for recovering *Gnathostoma spinigerum* third-stage larvae.

INTRODUCTION

In Thailand, *Gnathostoma spinigerum* is the causative agent of human gnathostomiasis (Daengsvang, 1981). The disease has also been reported in Japan, China, Malaysia, Indonesia, Philippines, Israel, and other countries where raw or pickled fish are consumed (Akahane *et al*, 1994; Ando *et al*, 1998; Rojekittikhun *et al*,1998). Clinical manifestations of human gnathostomiasis result from migration of immature larvae (L3s) and present as painful, pruritic swellings known as cutaneous larva migrans. Migration into internal organs (visceral larva migrans) can cause more severe manifestations, including death. In the latter case, the diagnosis should be made as soon as possible.

Presently, Western blot analysis - with antigen from parasite third-stage larvae - is used as the gold standard for diagnosis (Tapchaisri *et al*, 1991). For this process, antigen preparation following acid pepsin digestion is required (Tapchaisri *et al*, 1991). However, acid pepsin is expensive and not necessarily affordable for many laboratories in developing countries, including Thailand.

Due to the recent economic crisis in Thailand, an alternative substance that can be used instead of acid pepsin is of interest. Many natural products have been tested. Prawang *et al* (2002) proposed the use of pineapple juice as an alternative digestive agent for harvesting metacercaria of *Opisthorchis viverrini* from fish. Here, we tested another fruit (papaya) that has been known to contain enzymes with proteolytic effects (Morton, 1987). We investigated the feasibility of using papaya skin extract for digestion of swamp eel viscera to harvest infective stage larva of *Gnathostoma* spp and found that the papaya can be a good alternative to acid pepsin.

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MATERIALS AND METHODS

Preparation of papaya extract

The papayas (*Carica papaya)* were purchased from a local market at Klong Toey in Bangkok. The crude green papaya skin extract was prepared by using a juice extractor (National Model MJ 68 M). The extract solution was preserved at -20˚C, and adjusted to pH 2 with 1N HCl before use.

Digestion of swamp eel viscera

All eel livers were separated from other viscera, washed with tap water, then equally divided into two parts. The first part (10 grams) was digested with pepsin (1.5 % pepsin in water, adjusted to pH 2.0 with 1M HCl) 80 ml. The digestive process was performed in a water bath (37 ˚C, 4 hours) with frequent agitation. The digested liver was then washed repeatedly by sedimentation with 0.85 % NaCl solution. *Gnathostoma* L3 larvae were identified according to the characterization described by Daengsvang (1981), and counted by stereomicroscopy.

The second part of the eel's liver was digested by the prepared papaya skin extract in the same fashion as pepsin digestion. Digestion was performed in a water bath as mentioned earlier, *Gnathostoma* L3 larvae were identified and counted by stereomicroscopy.

Cultivation in BME culture medium

Some larvae were chosen and cultivated in BME culture medium (Gibco, Grand Island, NY, USA). Preparation of this medium was done by using one pack of BME powder (9.2 g) dissolved in 1 liter of distilled water and adjusted to pH 7.2-7.4 by $NaHCO_3$. Sterilization was done by 0.45 μm millipore membrane filtration.

RESULTS

With the equivalent weight of eel's liver used, the acid pepsin digestion yielded a higher number of larvae than papaya skin extract, 180 and 170 larvae, respectively, as shown in Table 1. Comparison between both solutions (one way ANOVA) shown that there was no significant difference ($p > 0.05$).

After 7-day cultivation in BME medium, almost all larvae obtained from pepsin digestion and papaya extract survived with survival rates of 93% and 94%, respectively (Table 2). There was no significant difference between the survival rates of larvae digested from either solution ($p > 0.05$; one way ANOVA).

DISCUSSION

The freshly prepared green papaya skin extract (crude) and 1.5 % acid pepsin solution yielded no statistical difference in number of third-stage larvae. The larvae obtained from both methods still survived when cultivated for 7 days. The 7-day period may be useful for those who want to cultivate the parasite for excretory antigen. This means that papaya extract can be effectively used for digestion of swamp eel viscera, similar to that of acid pepsin solution.

Table 1
The number of larvae obtained from pepsin digestion and papaya skin extract.

No. of test	1.5% acid pepsin			Papa skin extract		
	Encysted	Excysted		Encysted	Excysted	
		Active	Dead		Active	Dead
1	0	21	0	1	8	0
2	1	24	4	5	9	0
3	0	17	7	5	31	0
4	1	7	0	1	4	0
5	22	12	0	24	21	0
6	7	16	11	2	12	1
7	1	14	0	8	15	0
8	0	13	2	1	19	3
Total	32	124	24	47	119	4

Table 2
The survival of 3rd stage larvae in BME medium.

No. of tests	Number of larvae at start		Number of larvae on the 7th day	
	1.5% acid pepsin	Papaya skin extract	1.5% acid pepsin	Papaya skin extract
1	20	9	15	9
2	20	50	18	44
3	8	5	8	5
4	33	43	30	39
5	5	4	5	4
6	12	12	12	12
7	9	9	9	9
8	10	10	9	10
9	15	15	15	15
10	20	20	20	19
Total	152	177	141 (93%)	166 (94%)

The proteolytic action of green papaya skin extract is due to enzymes such as papain, chymopapain (Morton, 1987), or other proteolytic constituents. Chymopapain is the most abundant, but papain is twice as potent (Morton, 1987). Papain, the active enzyme in green papaya, dissolves dead surface skin cells without harming live ones (Beauty Deals, 2003). It was commonly used in the meat industry as a tenderizer. The greener the fruit, the more active the papain. This unique quality of papaya makes it an excellent natural meat tenderizer (Thai cuisine: Papaya, undated). Application of papaya's unique quality is currently used in pediatric burn units for wound dressings (Starley *et al,* 1999). Papaya latex contains not only papain and chymopapain but also papaya proteinase III (Zucker *et al,*1985). This study can not conclude that the proteolytic actions were due to only three papaya proteinases. It may be that other proteases such as aspartyl protease or cysteine protease play a role in this digestion. However, this study can conclude that crude green papaya skin extract may be an alternative to acid pepsin for the recovery of third-stage larvae of *Gnathostoma spinigerum* . The green papaya skin extract confers several advantages, such as year-round availability, easy and inexpensive preparation, and absence of toxicity.

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