

Feasibility Of Using Orange Juice For Digestion Of Swamp Eel Viscera For Harvesting Infective Stage Larva Of *Gnathostoma Spp.*

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Abstract

Gnathostoma spinigerum third stage larvae antigen is necessary for Western blot analysis in the diagnosis of *Gnathostomiasis*. Acid pepsin solution is required for digestion of eel's liver (*Fluta alba*) to yield the larvae for antigen preparation. Here, he reported the feasibility of using orange juice as an alternative to enzyme pepsin in harvesting *Gnathostoma spinigerum* third stage larvae. From the experimental digestion, it was found that we could recover larvae from orange juice. Thus, orange juice might be another choice for recovering *Gnathostoma spinigerum* third stage larvae.

INTRODUCTION

In Thailand *Gnathostoma spinigerum* is the major causative agent of human *gnathostomiasis*^{1,2}. The disease has also been reported in Japan, China, Malaysia, Indonesia, Philippines, Israel, and other areas where raw or pickled fishes are consumed^{3,4,5}. Clinical manifestations of human *gnathostomiasis* are resulted from migration of immature larvae (L3s) and presented as painful pruritic swelling known as cutaneous larva migrans. In addition, migration into internal organs (visceral larva migrans) can result in more severe manifestations including death. In the latter case, the diagnosis should be made as soon as possible.

According to the recent economical crisis in Thailand, an alternative substance that can be used in stead of acid pepsin is of interest. Many natural products are tested. Recently, Prawang et al⁶ proposed the use of pineapple juice as an alternative digestive agent for harvesting metacercaria of *Opisthorchis viverrini* from fishes. Here, we tested another fruit, orange, which has been known to have enzymes with proteolytic effects⁷ for this purpose. The feasibility of using orange juice for digestion of swamp eel viscera for harvesting infective stage larva of *Gnathostoma spp* was studied and we found that the orange can be another good alternative to acid pepsin.

MATERIALS AND METHODS

A) PREPARATION OF ORANGE JUICE

The oranges were purchased from a local market in Bangkok. The crude green orange juice was prepared as local cooking method.

B) DIGESTION OF SWAMP EEL VISCERA

All eel livers were separated from other viscera and washed with tap water. The part of eel's liver was digested by the prepared orange juice in the same fashion as done in pepsin digestion. Digestion was performed in a water bath as mentioned earlier, *Gnathostoma* L3 s were identified and counted by stereomicroscopy.

RESULTS

From the experimental digestion, it was found that we could recover larvae from orange juice. The digestion is similar to those performed by pepsin digestion in our laboratory.

DISCUSSION

Presently, Western blot analysis, which antigen from parasite third stage larvae is required, is used as gold standard⁸. For this process, antigen preparation following acid pepsin digestion is required⁸. However, acid pepsin is expensive and seems not affordable for many laboratory settings in the developing countries including Thailand.

In this study, the freshly prepared green orange juice (crude) and 1.5 % acid pepsin solution could yield third stage larvae. That means the extract can be effectively used for digestion

of swamp eel viscera, similar to that of acid pepsin solution. This unique quality of orange makes it an excellent natural meat tenderizer (Thai cuisine name: Som) This study can assure that crude green orange juice can be alternative to acid pepsin for the recovery of third stage larvae of *G. spinigerum*. Interestingly, the green orange juice confers several advantages such as all year round availability, easy and inexpensive preparation and non-toxic. Although this work is simple it remains originality, no previous experiment on this topic was reported. In addition, this new digestive technique can be useful for the practitioners in the developing countries with limited resource.

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