# Synergistic Anthelmintic Potency of Papaya-Pumpkin Seeds Against *Pheretima posthuma*

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## ABSTRACT

*Carica papaya and Cucurbita maxima* or respectively known as papaya and pumpkin have been used for treatment in many types of diseases for centuries. The crude aqueous extracts of both *C. papaya* and *C. maxima* seeds were assayed synergistically against adult earthworms, *Pheretima posthuma* (*P. posthuma*) for the evaluation of anthelmintic activity and compared with a standard drug or positive control, Albendazole. In this study, the extraction was carried out using boiling and evaporation methods. Different concentrations (60mg/ml, 40mg/ml, and 20mg/ml) of papaya-pumpkin seed extracts and Albendazole were tested on each petri dish containing two worms. Time of paralysis (P) and death (D) of worms were recorded and analysed. The result showed that the crude aqueous extracts of seeds at a concentration of 20mg/ml could exterminate the worms at the shortest time (111.50 ± 4.95 minutes) compared to Albendazole (112.50 ± 4.96 minutes). In conclusion, the combination of *C. papaya* and *C. maxima* seed extracts is a good option to be taken as alternative medicine in treating cases of intestinal worm infections.

Keywords: Anthelmintic, *Pheretima posthuma, Carica* papaya*, Cucurbita maxima,* synergistic

## INTRODUCTION

Helminthiasis is an invasion with one or more intestinal parasitic worms, such as roundworms, whipworms, or hookworms in humans and animal. About 80% of the worldwide population uses traditional medicine for primary healthcare (WHO, 2017). Nazneen et al. (2017) mentioned that plants have been used from primal to treat diseases of man and animals. Besides, a recent study performed by Patil et al. (2019) discovered that the use of natural products as anthelmintic has become a conventional therapy due to the wide spectrum of actions and fewer side effects to the body without problems of drug resistance. Therefore, this study aims to investigate the synergistic effect of papaya and pumpkin seeds for their appealed anthelmintic activity.

*Carica papaya*, commonly known as papaya, belongs to the *Caricaceae* family. It is described as a fast-growing, straight, usually unbanked tree or climber, 7-8m tall with copious latex, the trunk of about 20cm in diameter (Kiran et al., 2013). The seeds are covered by transparent gel and are attached to the hollow in the central part of the fruit by soft, thread-like tissue. There are several chemical materials reported in different parts of *C. papaya* (Islam et al., 2019). Papaya skin, pulp, and seeds contain a variety of phytochemicals such as ascorbic acid, riboflavin, and tannins. The fruit also has enzymes, including papain, lycopene, and polyphenols (Sengupta et al., 2013). A papaya plant possesses many medicinal values and has been prepared scientifically as an extract using various types of solvents and experimentally tested using a range of in vivo and in vitro. These include testing on anticancer, anti-inflammatory, antipyretic, antidiabetic, antioxidant, antibacterial, larvicidal, antifungal, and antimalarial, which required quite an in-depth study as more previous studies have been carried out (Asghar et al., 2016; Ningsih & Rejeki, 2018; Islam et al., 2019; Okigbo et al., 2019; Fasoyinu et al., 2019; Sasongko et al., 2019). Earlier studies reported that an aqueous extract of *C. papaya* seeds has inactivated bacterial activity of gram-positive and gram-negative bacteria that indicated the presence of broad-spectrum antimicrobial compounds (Kiran et al., 2013).

*Cucurbita maxima* belongs to *Cucurbitaceae* family of the *Cucurbitales* order. It is a sprawling annual herb that grows either prostate along the ground or climbs using tendrils. The fruit contains several to hundreds of flat oval seeds which may be garlanded or coloured. Some varieties contain edible and medicinal seed oil (Paris, 2010). There are limited studies done on chemical compositions of *C. maxima* plant as the researchers were more interested in the seeds of the plant. Pumpkin seeds contain four fatty acids which are palmitic, stearic, oleic, and linoleic acid and contain

enzymes such as phytosterols, urease, and lipase (Stevenson et al., 2007; Montesano et al., 2018). It has been proven in the study carried out by Chiej (2001) that the seeds are diuretic, tonic, and vermifuge. The aqueous *C. maxima* seed extracts have been proven to act as an anthelmintic (Sengupta et al., 2013; Ayaz et al., 2015).

## MATERIALS AND METHODS

### Sample Collection and Preparation of Extracts

The seeds of *C. papaya* and *C. maxima* were collected from the papaya and pumpkin purchased from a local market in Kuala Lumpur, Malaysia. The shade-dried seeds were placed into the oven at 55°C overnight for confirmation. Then, powdered seeds of *C. papaya* and *C. maxima* were boiled in distilled water separately. The suspensions were then filtered using four layers of Whatman® filter paper No.1. The resulting filtrates were then evaporated in the water bath at 50°C overnight to dryness and thus a brownish and yellowish semisolid mass of papaya and pumpkin seed extracts were obtained, respectively.

### Worm Collection

Adult earthworms (*P. posthuma*) were selected as experiment models due to their anatomical and physiological resemblance to the human intestinal roundworms. They were collected from moist soil areas at Puncak Alam, Selangor, Malaysia and washed with 0.9% normal saline to remove dirt. The earthworms selected were uniform in size, ranged from 6cm to 10cm in length.

### Sample Preparation

The extract of papaya and pumpkin seeds and albendazole were dissolved in distilled water to prepare three different types of concentrations (20mg/ml, 40mg/ml and 60mg/ml). Each extract was mixed in 1:1 ratio to evaluate the synergistic effect.

### Anthelmintic Activity

The anthelmintic activity assay was performed according to the method by Akter et al. (2014) with minor modifications. *P. posthuma* is easily available and suitable models for evaluation of anthelmintic activity due to the similarity of anatomical and physiological structures to roundworms of the human gastrointestinal tract (Husori et al., 2018). Seven groups of worms were made, each containing the approximately equal size of two adult worms. The albendazole (positive control) and extract a mixture of papaya and pumpkin seeds at different concentrations were applied to each group of worms. Meanwhile, distilled water was used as a negative control. Time of paralysis was reported when there was no movement of any sort could be detected except when the petri dish containing worms were shaken vigorously. Time of death was recorded after ascertaining that the worms neither moved when shaken vigorously nor dipped in the warm water of 50$^{o}$C.

## RESULTS

The results are expressed as the mean ± SD of two worms in each group. Comparisons have been made between standard drug against test groups in concentrations of 20mg/ml, 40mg/ml and 60mg/ml. A $p < 0.05$ was considered significant. The observations are shown in Table 1.

TABLE 1. *In vitro* anthelmintic activity of a combination extracts of *C. papaya* and *C. maxima* seeds compared with a standard drug (Albendazole). Values of time taken are expressed as mean ± standard deviation (SD). N in the table referring to total sample size (N=2) Mean value considered significantly different when $p< 0.05$.

| Groups | Concentration (mg/ml) | N | Time taken of paralysis (P) (min) | Time taken of death (D) (min) |
|---|---|---|---|---|
| Control (Distilled water) | - | | - | - |
| Aqueous extracts of *C.papaya* and *C.maxima* (1:1) | 20 | 2 | **46.65 ± 2.23** | **111.50 ±4.95** |
| | 40 | 2 | **36.97 ± 2.06** | **96.00 ± 8.49** |
| | 60 | 2 | **31.60 ± 3.44** | **68.50 ± 9.19** |
| Standard drug (Albendazole) | 20 | 2 | **56.91 ± 1.99** | 112.50 ± 4.96 |
| | 40 | 2 | **44.54 ± 3.54** | 100.00 ±4.24 |
| | 60 | 2 | **33.79 ± 0.47** | 75.00 ± 11.31 |

## DISCUSSION

The combination of papaya-pumpkin seeds aqueous extract showed significantly higher anthelmintic activity at all concentrations compared to the standard drug. The highest activity of seeds extract was observed at the concentration of 60mg/ml. The result of this study (Table 1) has clearly shown that the time for paralysis and death decreased with increasing concentrations of the seed extracts.

The in vitro anthelmintic activity of the aqueous extract could be described by the fact that several compounds like alkaloid, flavonoid, tannins, and papains that were present in both seeds might be responsible for the anthelmintic activity of the plants (Sengupta et al., 2013; Effendy et al., 2014; Asghar et al., 2016). A past study done by Sengupta et al. (2013) stated that the presence of tannins in both seeds enables them to bind to glycoprotein on the cuticles of the parasite and cause death, thus inhibited its polymerisation or assembly into microtubules. The combination of both *C. papaya* and *C. maxima* seed extracts possessed a higher anthelmintic effect than commercial drugs. Albendazole blocks the glucose uptake by the susceptible *P. posthuma*, thereby depleting the energy of the worms for survival as adenine triphosphate (ATP); thus, the worms become immobilised and die (Bogitsh et al., 2013). Besides, albendazole can cause degenerative alterations in the outer layer and intestinal cells of the worms by binding to the colchicine-sensitive site of tubulin, hence also inhibiting its polymerisation (Malik & Dua, 2020). From the results obtained, the time taken for death and paralysis decreases as the concentrations of the crude extracts increase. In this study, aqueous extracts were used instead of chemicals such as methanol or ethyl acetate. This is because a previous study done by Kiran et al. (2013) had proven that aqueous extract of *C. papaya* showed shorter time taken for paralysis and death compared to methanol extracts of *C. papaya*. The aqueous extract contains the whole compound of plant material compared to alcohol extraction which may have only some molecules and this depends on the solvent polarity. If the study was to identify and isolate secondary plant compounds, then alcohol should be used (Dhanani et al., 2017).

## CONCLUSION

From the above result, it can be concluded that a combination of aqueous extracts of *C. papaya* and *C. maxima* could be suggested as a potent alternative for synthetic anthelmintic drugs. This study observed that both papaya-pumpkin seeds gave the shortest time of anthelmintic even at the lowest concentration compared to a standard drug, albendazole. The phytochemical compound contained in both seeds was able to expel the worms in the human host. The availability of *C. papaya* and *C. maxima* seeds as a cost-effective drug may

decrease the cases of worm infections, especially among the rural community. However, further in vivo studies against different parasites of humans and other animals at different doses are needed to determine the potential of papaya-pumpkin seed extracts as an anthelmintic against gastrointestinal worms.

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