

Combined Effects of *Carica papaya* Seeds with Albendazole on Adult *Pheretima posthuma*

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ABSTRACT

Background: *Carica papaya* seed is commonly used as herbal remedy for intestinal worms. This study investigated the combined effect of *Carica papaya* seed extracts and albendazole on adult *Pheretima posthuma*.

Methods: Cold percolation method was used to extract bioactive compounds in dry powdered seeds of *Carica papaya* using water, ethyl acetate and petroleum ether as solvents. Individual extracts and combinations of the extracts with albendazole were assayed for anthelmintic activity in which the time of paralysis (P) and time of death (D) of the worms were determined.

Results: Aqueous extract gave highest extractive yield of 19.8% followed by the ethyl acetate extract at 17.2%. Moreover, the individual aqueous extract and that combined with albendazole paralyzed the worm within shorter time (41.00 and 42.00 minutes respectively) compared to the conventional drug albendazole alone (48.67 minutes). Conversely, Albendazole killed the worms faster than any other extract in just 1.18 hours. Albendazole combined either with water or ethyl acetate killed the worm at 24th hour. Combined albendazole with petroleum ether extract could not kill the worm even after 24 hours.

Conclusion: From this study, combining the *C. papaya* seed extracts with albendazole did not shorten paralytic and anthelmintic activity on adult *Pheretima posthuma*. *Carica papaya* seeds extracts should rather be used individually and best when extracted in water.

Key words: Antihelmintic, *Carica papaya*, Bioactive

INTRODUCTION

Globally, soil transmitted helminthes affect over 1.5 billion people and it accounts for loss of about 200,000 human lives annually¹. Whipworms (*Trichuris trichiura*), Roundworms (*Ascaris lumbricoides*), and Hookworms (*Necator americanus* and *Ancylostoma duodenale*) are helminthes of medical importance and are mainly transmitted through contaminated soil, water, food, and contact with animals' dung². They are responsible for infections and complications such as; weakness, loss of appetite, ³ reduced weight gain and decreased productivity, aggravating malnutrition, retarded growth and development, retarded cognitive development, anemia, abdominal pains and diarrhea in the affected host⁴. To date, the conventional

gold standard for the treatment of soil transmitted helminthiasis has been the use of Benzimidazole (Albendazole and Mebendazole) drugs⁵. However, with the outgrowth of Anthelmintic Resistance (AR) in the livestock industry due to years of mass drug administration and misuse of chemical anthelmintic drugs, soil transmitted helminthiasis is gradually re-emerging⁶. Besides, majority of patients (80%) in poverty stricken economies like Uganda, can hardly afford the conventional drugs hence depending mostly on herbal or medicinal alternatives⁷. *Carica papaya* (Linn.), commonly known as papaya is a fruit crop cultivated in tropical and subtropical regions, and is well known for its nutritional benefits and medicinal applications⁸. Several

studies have revealed the anthelmintic potentials of *C. papaya* on gastrointestinal worms.

For instance, a study by Wasswa and Olila⁹ revealed the effective dose (ED50) of *Carica papaya* seeds on *Ascaris suum* at 12.5 mg/ml. Likewise, in comparison to albendazole, *C. papaya* seeds was found to be three times more effective on adult *Pheritima posthuma*¹⁰ and mixed gastrointestinal nematodes¹¹. Still, no study has made an attempt to consider the effect of combining the papaya seed extract with a common drug Albendazole. Given the far limited number of anthelmintic drugs in market together with hardly new compounds, this study investigated the possibility of a combination treatment approach by combining papaya seed extracts with Albendazole (5-propylthio-2-benzimidazolecarbomate) on adult earth worms (*Pheritima posthuma*). This could contribute to the knowledge base for a sustainable alternative against soil transmitted helminthiasis.

METHODS

Test Worms and Control Drug

The study used adult *Pheritima posthuma* (earthworms) obtained from moist soils around Lake Victoria in Entebbe, Uganda (0.0436°N, 32.4418°E). The worms were authenticated at the Helminthology section of National Animal Disease Diagnostics and Epidemiology Centre (NADDEC), Entebbe, Uganda. The choice of the earthworms was based on their anatomical and physiological resemblance with the gastrointestinal roundworm parasites of human beings¹². Albendazole tablet (400 mg) manufactured by Beximco pharmaceuticals (Gazipar, Bangladesh) was used as a control drug.

Collection of plant materials

The fresh seeds were collected from ripe *Carica papaya* fruits and washed with clean water to remove dirt. The seeds were then dried in the hot air oven (Memmert, Schwabach, Germany) at temperature of 50°C for 24 hours and later coarsely powdered using a homogenizer prior to extraction.

Extraction procedure

Dry powdered seeds was extracted by cold percolation method according to Parekh & Chanda et al,¹³ with minor modifications. Petroleum ether, ethyl acetate and distilled water were used as solvents to maximize phytochemical extraction due to varying polarity. Briefly, 5 g of dried powder was added to a 250 ml conical flask and Petroleum ether was added to the conical flask (Phyrex, Werthiem, Germany) with sample to the 100 ml mark, the flask was sealed with aluminum foil, and kept on a rotary shaker (Ika, Staufen, Germany) at 120 rounds per minute (rpm) for 24 hours.

The solution was filtered through eight layers of muslin cloth, centrifuged (Beckman, Indianapolis, USA) at 5000 rpm for 15 minutes and the supernatant was collected and the residue was evaporated to dryness using an oven (Memmert, Schwabach, Germany). Each of the dry residue was transferred to 100 ml of individual solvent (Distilled Water and Ethyl Acetate) and was kept on a rotary shaker at 120 rpm for 24 hours. After 24 hours, the solution was filtered through eight layers of muslin cloth, centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. Each of the filtered solution was left on petri dishes to evaporate. Extractive yield of each extract was calculated and later each of the three extracts were stored at 4°C for anthelmintic assay.

Anthelmintic assays

The individual and combined anthelmintic activity of *Carica papaya* seed extracts was carried out as described by Ajaiyeoba et al.¹⁴ with minor modifications. Extracts, control drug and their respective combinations were administered through contact. Two worms were fully immersed in a 9 cm radius petri dish containing 10 ml of each of the extracts described above, or a combination of the extracts and albendazole. For this study, a uniform concentration of 10 mg/mL was empirically used. This was done in triplicates for all the samples and the time for induced paralysis (P, in minutes) was taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Time of death of worms, if death occurred (D, in minutes) was recorded after ascertaining that worms neither moved when shaken vigorously nor when pricked with a needle. Albendazole (10 mg/ml) was included as reference/control drug, while distilled water was included as the negative control.

Statistical analysis

Tableau Software v2019.4 was used in data presentation. All experiments were repeated at least three times and the Standard Error of the Mean (SEM) determined. The means were compared using SPSS software v16.0 in which a one-sample T-test was performed to determine the significance of the anthelmintic potentials of *Carica papaya* seeds.

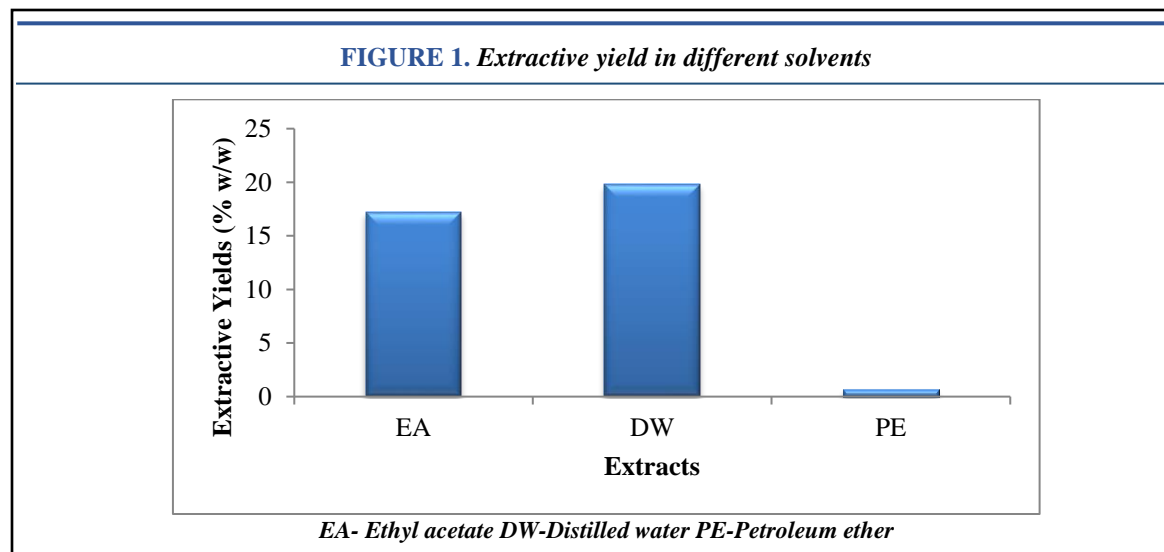
Ethical consideration

Ethical approval and permission to conduct this study was obtained from the Bugema University Research Ethics Committee, Kampala, Uganda (Ref. BU-REC/2019/U09).

RESULTS

Extractive yields

The extractive yield of *Carica papaya* seeds across three different solvents is presented in **Figure 1**.



Distilled water presented with the highest yield (19.8%) as compared to ethyl acetate and petroleum ether extracts. Ethyl acetate gave about 17.2% while petroleum ether presented with the least extractive yield (1%).

Anthelmintic Activity assay

The anthelmintic potentials of 10 mg/mL *Carica papaya* seed extracts and extract-drug combinations are presented in **Table 1**. The mean paralysis time for distilled water extract and its combination with Albendazole were found to be shorter than that of the control drug Albendazole ($p < 0.05$), indicating that they are more effective in paralyzing the worm than Albendazole alone. Even though, individual ethyl

acetate and its combination also had shorter paralysis time than Albendazole, this was not statistically significant ($p > 0.05$) and the rest of the extracts were less promising compared to the control drug (Mean Paralysis Time $> 46.67 \pm 4.16$). With respect to the time of death, neither individual petroleum ether extract nor its combination could kill the worms even after 24 hours whereas, the ethyl acetate extracts (individual extract) could kill the worms within a few hours (2.10 ± 0.10). Surprisingly, combining *C. papaya* extract with Albendazole delayed anthelmintic activity by an average of 12 minutes ($r = 1.00, p = 0.001$). A similar trend was observed in distilled water extract and its combination with albendazole.

TABLE 1. Anthelmintic activity of *Carica papaya* seed extracts.

Test sample	Paralysis Mean Time (Minutes, SD)	Death Mean Time (Hours, SD)
EA	44.67±2.31	2.10±0.10 [†]
DW	41.00±1.73 [†]	24.00±0.19 [†]
PE	107.33±7.37 [†]	No death after 24 Hours
EA+ Albendazole	46.67±1.55	24.00±1.00 [†]
DW+ Albendazole	42.00±1.73 [†]	24.00±0.62 [†]
PE+ Albendazole	100.33±4.04 [†]	No death after 24 Hours
Albendazole	48.67±4.16	1.18±0.10 [†]

EA- Ethyl acetate extract, DW-Distilled water extract, PE-Petroleum ether extract, †p < 0.05. All values expressed as Mean ± Standard Error of Mean (n=3)

DISCUSSION

Here we found that combining individual extract of *Carica papaya* seeds with Albendazole decreases their anthelmintic activity on adult *Pheritima posthuma*. One inherent weakness of this study is that a fixed dose (10 mg/mL) was used instead of varying the concentration of the extracts. Besides the extract used was crude in comparison to pure control drug, Albendazole of the same concentration and this could have

affected the activity of the bioactive compounds in the test sample. However, the cold percolation approach of extraction enabled maximum extraction of bioactive compounds across a wide range of polarity and as well mitigated the loss of the volatile components on heating. Across all individual extracts, the time for paralysis and death of *Pheritima posthuma* in this study (41.00-107.33 minutes and 2.1-24 hours respectively) were longer than in previous study¹⁵ with paralysis and death

time of <35 minutes and < 1 hour respectively. The variation in paralysis and death time in this study could be due to lower concentration, 10 mg/ml as compared to the 25-100 mg/ml concentration range in the above-mentioned comparative study. Similarly, a number of other documented studies have demonstrated the anthelmintic activity of *Carica papaya*,^{9,10,16-19}. However, this study advanced to report the effect of combining the individual extracts with control anthelmintic drug, albendazole. *Carica papaya* seeds contain several bioactive compounds with the main ones being enzyme papain and benzylisothiocyanate.²⁰ In addition, the seeds are rich with other phytochemicals like tannins, alkanoids, oxalates and trypsin inhibitors²¹. The presence of tannins in *Carica papaya* seeds is the chief reason for its anthelmintic potentials, possibly because tannins can bind onto glycoproteins on the cuticle of the worm to induce necrosis thereby causing death of the worm²². Thus, suggesting the possible use of extract of such seeds as de-wormers. However, the individual extract in combination with control drug showed decreased activity. This could be due to a number of reasons ranging from target incompatibility as well as inhibitory effects²³. The study thence suggests that seeds of *Carica papaya* should be used individually if effective action against the worms is to be achieved. Furthermore, it is needful that anthelmintic bioactive compounds be purified, and structure elucidated for an accurate evaluation of antagonistic activity and its mechanism of action. Toxicity studies of the individual seed extract should as well be executed to establish the safety indices of such extracts.

CONCLUSION

From this study, combining the *C. papaya* seed extracts with albendazole did not shorten their paralytic and anthelmintic activity on adult *Pheritima posthuma*. *Carica papaya* seeds extracts should rather be used individually and best when extracted in water.

Conflict of Interest

The authors declare no conflict of interest related to this study

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Peer Reviewed

Competing Interests: None declared.

Received: 14 Jan 2020; Accepted: 23 Apr 2020.

Cite this article as: Muunda M, Musubire JB, Nassali G, Shumbusho E, Atim V, Ssematimba P, Kebirungi P, Atwijukire E, Okella H. Combined Effects of *Carica papaya* Seeds with Albendazole on Adult *Pheritima posthuma*. *E Afr Sci*. 2020;1(2):88-91. <http://doi.org/10.24248/EASci-D-20-00002>

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