UNIVERSITI PUTRA MALAYSIA

IN VITRO ANTHELMINTIC ACTIVITY OF PAPAYA LEAVES (*CARICA PAPAYA*) CHLOROFORM EXTRACT AGAINST THE THIRD-STAGE LARVAE OF STRONGYLES FROM SHEEP

AISYAH BINTI AHMAD PAUZI

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AISYAH BINTI AHMAD PAUZI

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "*In vitro* anthelmintic activity of papaya leaves (*Carica papaya*) chloroform extract against the third-stage larvae of strongyles from sheep", by Aisyah Binti Ahmad Pauzi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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DR SITI ZUBAIDAH RAMANOON

DVM (UPM), MSc (Guelph)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

_____

DR WAN MASTURA SHAIK MOHAMED MOSSADEQ

DVM (UPM), MSc (Guelph), PhD (UPM)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to the One Almighty God, who had created me and made all things possible,

To my family,

Father

Mother

Sisters

And to all my teachers who have committed themselves towards the noble cause of education.

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It is with deepest appreciation and gratitude that I thank Allah and all those who have made this project paper a reality.

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LIST OF ABREVIATIONS

%	Percent
µL	Microlitre
df	Degree of freedom
g	Grams
kg	Kilograms
mg	Miligrams
mg/ml	Milligram per mililitres
ml	Millilitres
CPE	*Carica papaya e*xtract
DMSO	Dimethyl sulfoxide
DVS	Department of Veterinary Services
FAO	Food and Agriculture Organization
GI	Gastrointestinal
KW	Kruskal-Wallis
L3	Third stage larvae
PGE	Parasitic gastroenteritis

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

AKTIVITI ANTELMINTIK *IN VITRO* EKSTRAK KLOROFOM DAUN BETIK (*CARICA PAPAYA*) KE ATAS LARVA STRONGIL PERINGKAT KETIGA DARIPADA BIRI-BIRI

Oleh

Aisyah Binti Ahmad Pauzi

2016

Penyelia: Dr. Siti Zubaidah Ramanoon

Penyelia bersama: Dr. Wan Mastura Shaik Mohamed Mossadeq

Gastroenteritis berparasit adalah salah satu punca utama kerugian ekonomi dalam amalan ruminan kecil. Helmintiasis telah menyebabkan morbiditi teruk dan kematian setiap tahun dalam industri ruminan kecil di Malaysia, dimana antelmintik kimia telah digunakan dalam rawatan dan pencegahan. Namun, penggunaan antelmintik kimia yang kerap dan sembarangan telah menyebabkan masalah ketahanan antelmintik dalam populasi ruminan kecil. Kajian ini bertujuan untuk menilai kesan antelmintik ekstrak klorofom daun betik *(Carica papaya*) (CPE) pada larva strongil peringkat ketiga (L3) daripada biri-biri. Melalui kultur tinja, L3 dituai selepas 7 hari. Seratus L3 diletakkan dalam setiap satu ceper petri bagi lima kumpulan (iaitu CPE pada tiga kepekatan yang berlainan, satu levamisol dan satu kawalan negatif) yang mempunyai enam ceper petri bagi setiap kumpulan. Kadar kematian L3 diperhatikan pada jam ke-

2, 4, 6, 24 dan 48. Hasil kajian mendapati bahawa CPE pada kepekatan 7.5, 10.0 dan 12.5 mg/ml, menunjukkan aktiviti antelmintik ketara pada L3 dengan kadar kematian sehingga 99% (KW = 115.559, df = 4, $p < 0.05$). Kesemua L3 mati pada kepekatan levamisol 10mg/ml seawal jam ke-2 pemerhatian. Kesimpulannya, CPE berpotensi untuk digunakan sebagai agen antelmintik alternatif dari sumber herba pada masa akan datang. Walau bagaimanapun, kajian secara *in vivo* diperlukan untuk menentukan kesan antelmintik CPE dalam biri-biri.

Kata kunci: daun betik, *Carica papaya*, ekstrak klorofom, strongil, L3, biri-biri

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

***IN VITRO* ANTHELMINTIC ACTIVITY OF PAPAYA LEAVES (*CARICA PAPAYA*) CHLOROFORM EXTRACT AGAINST THE THIRD-STAGE LARVAE OF STRONGYLES FROM SHEEP**

By

Aisyah Binti Ahmad Pauzi

2016

Supervisor: Dr. Siti Zubaidah Ramanoon

Co-supervisor: Dr. Wan Mastura Shaik Mohamed Mossadeq

Parasitic gastroenteritis (PGE) is one of the major causes of economic losses in small ruminant practices. Helminthiasis has been reported to cause severe morbidity and mortality annually in the small ruminant industry in Malaysia, whereby chemical anthelmintic has been used for treatment and prevention. However, frequent and indiscriminate use of chemical anthelmintic has resulted in resistance problem in the small ruminant population. This study aimed to evaluate the anthelmintic effect of papaya leaves (*Carica papaya*) chloroform extract (CPE) on the third-stage larvae (L3) of strongyles from sheep. The L3 larvae of strongyles from sheep were harvested 7 days post faecal culture technique. Six petri dishes containing one hundred L3 per perti dish were used for each of the CPE treatment groups, one Levamisole and one negative control group. The mortality rate of these larvae was observed at 2, 4, 6, 24

and 48h. Results showed that CPE at the concentrations of 7.5, 10.0 and 12.5 mg/ml, exerted significant anthelmintic activity against L3 with a mortality rate up to 99% (KW=115.559, df= 4, $p < 0.05$). Levamisole (10mg/ml) induced 100% L3 mortality at the second hour of observation. In conclusion, CPE could potentially be used as an alternative herbal anthelmintic agent in the future. However, further *in vivo* research is needed to determine the anthelmintic effect of CPE in sheep.

Keywords: papaya leaves, *Carica papaya*, chloroform extract, strongyle, L3, sheep

1.0 INTRODUCTION

1.1 Background

The small ruminant population in Malaysia specifically sheep population has increased over time according to the Department of Veterinary Service (DVS). The sheep population has increased from 131, 293 in 2012 to 141,918 sheep in 2013. However, helminthiasis still remains as the major problem and have been identified to cause severe mortality and morbidity in the small ruminant industry in Malaysia (Fatimah *et al.*, 1985). In addition, helminthiasis has become a major public health and economic importance in humans and animals in the tropics. About 60 – 80% of the world population was estimated to be affected by helminthiasis with a vast majority of the cases occurring in developing countries (Farnsworth, 1988).

Anthelmintic protocol has been introduced to combat this problem ever since. However, frequent and indiscriminate use of these anthelmintics has caused anthelmintic resistance problem (Klauck *et al.*, 2014). Thus, alternatives to the current commercialized anthelmintics are urgently required.

A study by Wasswa *et al* (2006) showed that some plants used in ethno veterinary medicine could be of value in the treatment of helminthiasis. Its leaves and fruits are important in pharmaceutical and industrial applications as they produce several proteins and alkaloids that are beneficial in those industries (El Moussaoui *et al*., 2001). Herbal products such as neem, turmeric, papaya extract (Odhong *et al.*,

2014) and *Leucaena leucocephala* (Oliviera *et al*., 2011) for example, have been used to combat helminthiasis. A study conducted by Ferreira *et al*., (2013) showed that *Annona muricata* L. aqueous extract exerted a significant anthelmintic activity against *Haemonchus contortus*.

The papaya plant in particular, has been known to be used for numerous reasons such as an anti inflammatory, wound healing, allergies, improve cardiovascular system and is able to lower the blood cholesterol levels as it is a good source of fibre (Aravind *et al*, 2013).

The papaya plant (*Carica papaya*) also known as paw paw, originated from the lowlands of Eastern Central America from Mexico to Panama (Nakasone & Paul, 1998). Ever since papaya was introduced in Malaysia, it was cultivated for its fruit. The total production of papaya was estimated to be 40,000 tonnes in 1993. Malaysia was ranked the seventh position as a papaya-producing country (FAO, 1993). The industry has been developing ever since as there is a rise in export earnings from RM 3.3 million in 1985 to RM 21 million in 1990 (Mukhtiar, 1994).

The fact that papaya plant in Malaysia is widely available and that previous studies have indicated the potential of papaya leaf as an anthelmintic (Odhong *et al*. 2014) and local farmers may prefer a plant-based anthelmintic for treatment of GI parasites in their animals, therefore, more investigation into the anthelmintic properties of the plant is of interest.

1.2 Justification

A series of Malaysian studies have shown that resistance of GI parasites to available anthelmintic drugs is an issue within the small ruminant industry in Malaysia. In addition, studies on the use of papaya leaves or extracts as an anthelmintic agent is limited. Therefore, this study was conducted to provide alternative solution to help farmers control helminthiasis problems in their farms.

1.3 Objectives

This study was conducted to determine the anthelmintic effect of papaya leaves (*Carica papaya*) chloroform extract (CPE) on the stage 3 larvae (L3) of strongyles from sheep and to determine the concentration of CPE required to kill the L3 of strongyles from sheep.

1.4 Hypothesis

The papaya leaves (*Carica papaya*) (CPE) would significantly induce larvicidal effect on L3 of strongyles from sheep.

2.0 LITERATURE REVIEW

2.1 Strongylida in Sheep

Strongylida is composed of four superfamilies namely Strongyloidea, Trichostrongyloidea, Ancylostomatoidea and Metastrongyloidea (Dwight, 2009). The life cycle of the Strongylida is rather direct with free-living microbivorous first and second larval stage and infective larval stage (Figure 1) and the locations where the strongyles are in the body of a sheep (Figure 2). The female of all the superfamilies will lay typical strongyle eggs with smooth surface, ellipsoidal shells that contain an embryo of morula stage and being laid and passed out with the faeces of the infected hosts. The eggs will then develop in the environment into the third larvae stage which is the infective stage. These infective stage larvae are being exposed to the susceptible host through feeding. These opportunistic parasites may cause clinical signs in the infected host such as watery diarrhoea and also pathologic changes in the alimentary tract (Mark, 2014).

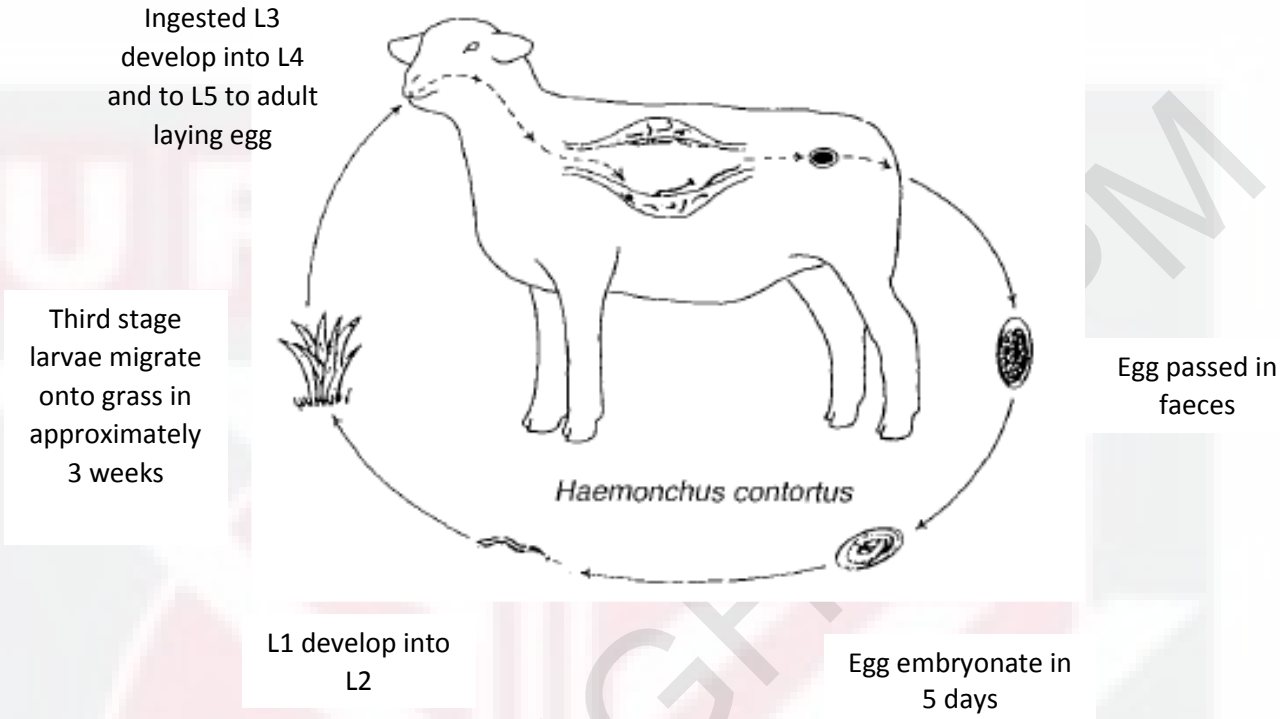

Figure 1. General Strongyle Life Cycle diagram (adopted from Foreyt W.J. 2001. Veterinary Parasitology Reference Manual Fifth Edition)

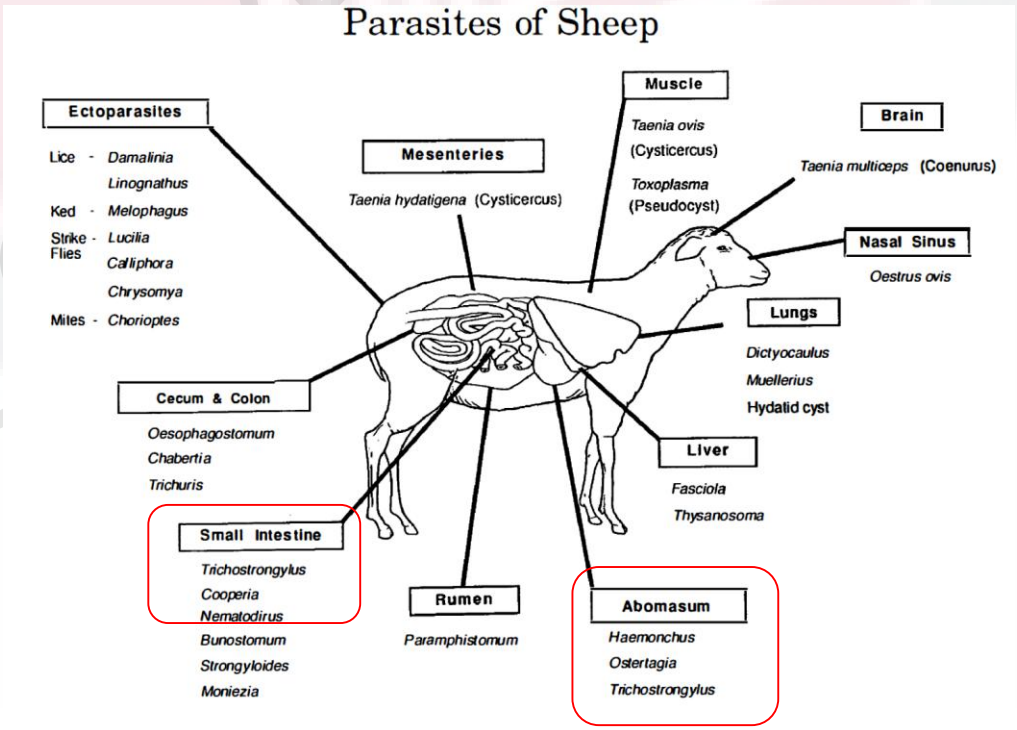

Figure 2. Locations of strongyle found in sheep (Adapted from Foreyt W.J. 2001. Veterinary Parasitology Reference Manual Fifth Edition)

2.2 *Carica papaya* (papaya)

Carica papaya belongs to the family Caricaceae and is a dicotyledonous and polygamous plant which has both male and female flowers on the same plant (Bennet *et al.*, 2005). *Carica papaya* is the most essential species within the Caricaceae family, as the plants are being cultivated widely for consumption as a fresh fruit or some other food products (Villegas, 1997). The papaya tree is widely planted in Malaysia. However, the use of this plant was limited to only as a food source. Despite having a numerous benefits in our daily lives, full utilisation of papaya plant is not being practiced.

Papain is a particularly important proteolytic enzyme that is produced in the milky latex of green, unripe papaya fruits and has been shown to have medicinal properties (Aravind *et al,* 2013). In the wild, papain may be associated with protection from frugivorous predators and herbivores (El Moussaoui *et al*., 2001). Commercially, papain has varied industrial uses in the beverages, food and pharmaceutical industries including in the production of chewing gums, tenderising meat, drug preparations and for various digestive problem (Villegas, 1997). In addition to papain, phytochemical analysis of different extracts showed presence of alkaloids, tannins, steroid and quinones (Bauri *et al*., 2015)

The ethanolic extract of papaya leaves has the highest content of alkaloids and tannins compared to the methanolic or chloroform extract; whereas n-Hexane has the highest content of steroids and chloroform extract showed the highest levels for both steroids and quinones (Juárez-Rojop *et al*., 2014).

2.3 Anthelmintic Usage

Anthelmintics are divided into several classes; benzimidazoles probenzimidazoles, salicylanilides and substituted phenols, imidazothiazoles, tetrahydropyrimidines, organophosphates, macrocyclic lactones and several newly introduced anthelmintics (Jozef *et al.,* 2014). Several classes of anthelmintics impair the cell structure, integrity or metabolism of the helminths such as benzimidazole and probenzimidazole which inhibit tubulin polymerization, whereas salicylanilides and substituted phenols groups act as uncouplers of oxidative that will cause the death of the larvae.

According to Chandrawathani (2003), over the past 20 years, small ruminant helminths have primarily been controlled by frequent anthelmintic use which resulted in severe anthelmintic resistance to benzimidazoles, avermectins, levamisoles and salicylanilides. Carmichael *et al.,* (1987) reported resistance to all groups of anthelmintics including the latest broad-spectrum anthelmintic, ivermectin. A survey by Dorny *et al.,* (1993) on 96 goat farms in Malaysia indicated that benzimidazole resistance was present on 36% of the premises. Khadijah *et al*., (2006) reported that 18 small ruminants private farms in Peninsular Malaysia showed althelmintic resistance to salicylanilides and closantel whereas 13 farms showed resistance to oxfendazole, 8 farms showed resistance to imidazothiazoles and 4 farms showed resistance to moxidectin. The reports thus raised concern on the status of anthelmintic resistance.

Therefore, the need to find other althelmintic alternatives that could combat helminthiasis and at the same time are cheap and economical to be produced in

mass quantities is crucial to help the small-scale small ruminant farmers in reducing the animal mortality and morbidity number in their farms due to helminthiasis. Hence, this study was conducted to search for a new solution to solve the anthelmintic resistance problem as papaya plant has shown a potential to be used as an anthelmintic alternative.

3.0 MATERIALS AND METHODS

3.1 Faecal sample, culture and harvesting L3

Faecal samples were collected from 22 sheep from a farm known to have GI parasitic problems in Hulu Langat Selangor. The faecal samples were cultured by faecal culture method for 7 days to obtain the L3. The faecal culture was left at room temperature and distilled water was sprinkled daily to maintain the moisture content of the faeces. On the 8th day, the larvae were harvested into a plastic test tube using the modified Baermann technique. The harvested solution was left to sediment and the supernatant was removed to obtain the larvae stock solution. The larvae stock solution was then kept in a plastic test tube and refrigerated at 26˚C until use. The volume of the stock solution required to obtain 100 larvae per petri dish was calculated by calculating the average volume of the stock solution needed to obtain 100 larvae which was 200µL (Norisal, 2015).

3.2 Collection and processing of plant material

The papaya leaves (*Carica papaya*) were collected from a papaya plantation in Mantin, Negeri Sembilan to obtain a total of 2.6 kg of leaves. Only young leaves were harvested, avoiding any shoots and mature leaves. A sample of the leaf was sent to the Herbarium Unit, Faculty of Forestry, Universiti Putra Malaysia for identification and verification. The leaves were cleaned using a damp cloth to remove dirt on each side. The cleaned leaves were then dried at room temperature (37˚C) for 3 days and then oven dried at 40˚C to remove excess moisture.

3.3 Preparation of the chloroform extract (CPE)

The large veins of the dried leaves were removed manually to obtain only the leafy part. The leaves were then grinded using 1mm-sized blade blender to obtain 200g of powdered papaya leaf. One hundred grams of the papaya leaf powder was soaked in 1L of 100% chloroform to 1:10 (w/v) and air dried for three days at room temperature. The suspension was later filtered using Whattman paper no. 1, three times before it was evaporated using the rotary evaporator at 40˚C to obtain 6g of papaya leaves extract. The extract was then dried in the fumigation hood for 2 days to remove any remnants of chloroform and then refrigerated at 20˚C until further use.

3.4 Preparation of diluents and CPE

Dimethylsulfoxide (DMSO) was mixed with deionised water to a concentration of 0.01% and kept at room temperature until use. The diluent was mixed with the dry extract to a concentration of 7.5, 10.0 and 12.5 mg/ml.

3.5 Experimental design

One hundred L3 from the stock solution were placed in petri dishes with CPE and the diluent (7.5, 10 mg and 12.5 mg/ml), Levamisole (10 mg/ml) and 0.01% DMSO solution. There were 5 groups (with six petri dishes in each group containing 100 L3 per dish): Groups: 1 – CPE 7.5 mg/ml; 2- CPE 10 mg/ml; 3-CPE 12.5 mg/ml; 4-Levamisole (positive control); and 5 – deionized water with 0.01% DMSO (negative control).

3.6 Assessment of the Anthelmintic Activity

The mortality of the L3 in all petri dishes from all 5 groups was recorded at the 2^{nd}, 4^{th}, 6^{th}, 24^{th} and 48^{th} hours post challenge. L3 was dead when there was no motility; whereas coiled and movable larvae were considered alive. All observations were done using stereo microscope.

3.7 Statistical Analysis

The data were tabulated in Microsoft Excel including the number of active and dead L3 for each time of observation (2, 4, 6, 24, 48 hour) at CPE concentration used (7, 10 and 12.5 mg/ml). Results were expressed as percentage (%) inhibition of larval motility calculated using the formula:

$$\text{Percentage inhibition (\%) of L3 mortality} = \left[\frac{\text{Number of dead L3}}{\text{Total number of L3 counted}}\right] \times 100$$

The L3 mortality percentages for all five groups were calculated and expressed in mean $\pm$ standard error of the mean (Mean $\pm$ SEM). Data of L3 mortality for all groups were inspected for normality using Shapiro-Wilk test whereby non-normal distribution was found ($p<0.05$). Therefore, non-parametric tests were used by applying Kruskal-Wallis and Mann-Whitney procedures to identify groups that have contributed to the significant effect. A p-value of <0.05 was considered statistically significant. All statistical tests were done in IBM SPSS Statistical Software for Social Sciences ver. 22. The results from all CPE treated groups were compared with the positive and negative controls. This study was conducted at the parasitology lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang between 11 Jan – 14 Feb 2016.

4.0 RESULTS

4.1 The *in vitro* anthelmintic effect of CPE on L3

Throughout the observation period, the mean percentage of L3 mortality increased with increasing concentrations of CPE (7.5, 10.0 and 12.5 mg/ml) used with a mortality rate of 63.57%, 69.33 and 79.47%, respectively (Table 1). The negative control group showed the lowest mortality rate (0.9%) whereas the highest moratlity rate was observed for the levamisole group (100%). The mean rank percentages of larvae mortality were 69.4%, 72.4%, and 86.2% at 7.5, 10.0, and 12.5 mg/ml CPE concentrations, respectively (Table 2). Kruskal-Wallis, H test showed no statistical significance difference of the L3 mortality between the CPE, levamisole, and negative control groups, χ^2 (4) = 115.559, p = 0.000.

Group	Concentration (mg/ml)	N	Mean ± SEM*
1	7.5	6	63.57 ± 5.005^{a}
2	10.0	6	69.33 ± 3.549^{a}
3	12.5	6	79.47 ± 2.647^{b}
4-Negative control	0.01% DMSO + Deionised water	6	0.90 ± 0.139^{c}
5-Positive control	0.01% DMSO + Levamisole (10mg/ml) + Deionised water	6	100^{d}

*Means with different superscripts differ significantly (p<0.05)

Table 1 The mean ± standard error of the mean (SEM) of CPE at 7.5, 10.0 and 12.5 mg/ml concentration on L3 mortality (%) against time (hours).

Group	Concentration (mg/ml)	N	Mean Rank
1	7.5	30	69.4
2	10.0	30	72.4
3	12.5	30	86.2
4-Negative control	0.01% DMSO + Deionised water	30	15.5
5-Positive control	0.01% DMSO + Levamisole + Deionised water	30	134.0
Total		150	

Table 2 The mean rank p of CPE at 7.5, 10.0 and 12.5 mg/ml concentration on L3 mortality (%) versus time (hours).

The percentage of L3 mortality against time is shown in Figure 2. CPE at all concentrations tested induced L3 mortality at an increasing percentage. CPE at a concentration of 7.5 mg/ml induced an increasing rate of L3 mortality between 18% (2 hours) and 98% (24 hours). Furthermore, CPE 10 mg/ml produced 48% to 98% L3 mortality at 2 hours to 48 hours observation period. The percentage of larval death continued to rise as the concentration was increased to 12.5 mg/ml with 61% to 99% larval death at 2 hours and 48 hours, respectively. Nevertheless, levamisole produced 100% L3 mortality within 2 hours of challenge. Based on Mann-Whitney test, there was significant difference in the percentage of L3 mortality and the CPE concentration for 7.5 mg/ml and 12.5 mg/ml ($U= 303.5$, $p= 0.030$), and 10.0 mg/ml and 12.5 mg/ml ($U= 305.5$, $p= 0.033$). However, the difference of L3 mortality at 7.5 mg/ml and 10.0 mg/ml was not different statistically ($U= 413.5$, $p=0.589$).

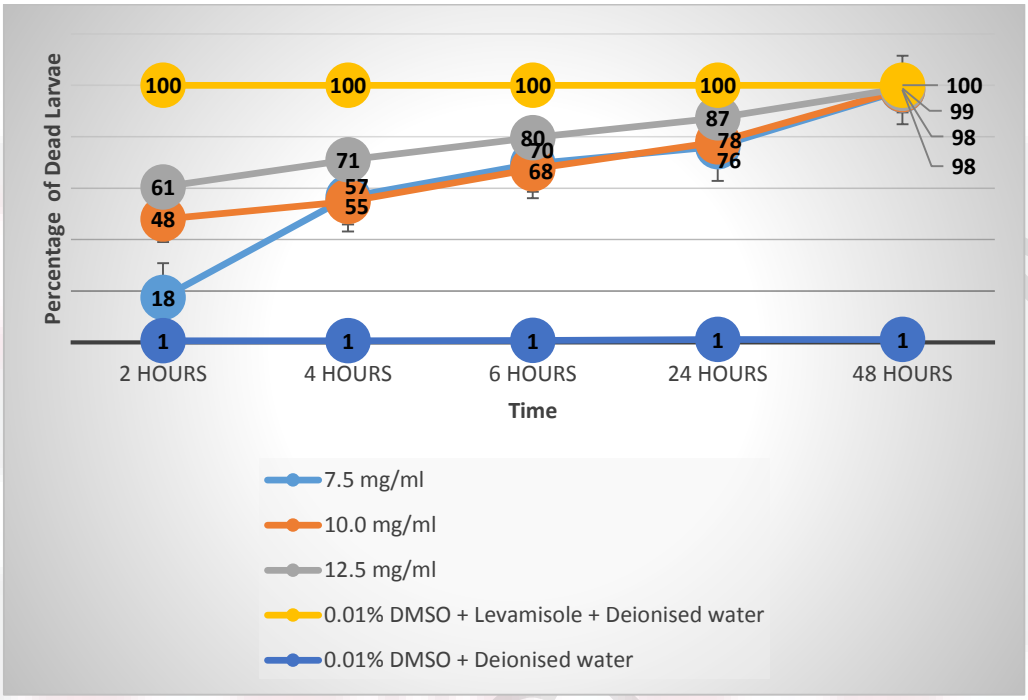

Figure 3 Effect of papaya leaves chloroform extract (CPE) on the L3 mortality (%) over time (hours)

5.0 DISCUSSION

Results from this study indicated that papaya leaves chloroform extract demonstrated larvicidal effect on L3 of strongyles cultured from the faeces of sheep. Also, the highest L3 mortality rate was recorded at CPE 12.5 mg/ml within 48 hours.

The larvicidal effect of CPE may be due to the activities of primary bioactive compounds such as benzyl isothiocyanate and papain (Kalaiyarasi *et al.*, 2014), and secondary metabolites that are present in the *Carica papaya* leaves (Bauri *et al*, 2015). A study conducted by Kermanshai *et al.*, (2001) showed that benzyl isothiocyanate acts as the chief anthelmintic agent that caused toxicity to helminth. Benzyl isothiocyanate produces larvicidal effect by inhibiting the energy metabolism and affecting the motor activity of larvae (Bauri *et al*., 2015). The depletion of energy caused exhaustion of the larvae and eventually death. Papain, the second primary metabolite found in this plant, causes larvicidal effect by digesting the cuticles of larvae due to its high proteolytic activities that brings about the death of larvae (Bauri *et al*., 2015). Other secondary metabolites that are present in the papaya leaves are saponins, tannins and phenols (Bauri *et al.*, 2015). Saponins act by causing the death of the larvae by affecting the permeability of the cell membrane of larvae which causes vacuolisation and disintegration of teguments and thus disrupt the homeostatic condition of the larvae and eventually cause the death of the larvae (Conrado *et al,* 2014). Tannins and phenols act synergistically by interfering with energy generation by uncoupling oxidative phosphorylation, binding to free protein of gastrointestinal tract as well as to glycoprotein on the cuticles, eventually causing exhaustion of the larvae and finally death of the larvae (Bauri *et al*., 2015). In summary, the synergistic

effect of these bioactive compounds and metabolites will eventually cause the reduction in the number or larvae by causing death to the larvae.

In the present study, the highest percentage of L3 mortality was observed at 48 hours post challenge. Interestingly, an *in vivo* study of papaya leaf extract in mice infected with larvae by Shaziya *et al*., (2012) also showed a decrease in number of larvae at 12 hours after infection and a sudden decrease in larval recovery at 18 hours, with a maximum larval reduction observed at 24 hours. However, our study showed that, longer time was required to induce 100% larvae death *in vitro*. The difference might be due to the different environmental conditions that may affect the survival rate of the larvae.

On the other hand, CPE at 12.5 mg/ml induced anthelmintic activity at the fastest rate compared to other CPE concentrations used. According to Shaziya *et al.,* (2012) the amount of major bioactive compounds and the secondary metabolite present in the extract influences the duration required to induce the larvicidal effect. These bioactive compounds acts similarly like other anthelmintics, by either starving larvae to death or paralyzing them. Paralysis and temporary loss of the ability to maintain position in the gut will eventually cause death in these larvae (Lakhsmi *et al*., 2012).

In addition, worms have no means of storing energy and thus any disruption in this process results in energy depletion. Furthermore, interference with larvae's feeding activity for a duration of 24 hours or less is sufficient to kill most adult parasites (Shaziya *et al*., 2012). In summary, the larvicidal effect of CPE could be due to the content of bioactive compounds and secondary metabolite that are present in the leaves

that mimic the mechanism anthelmintics drug in causing the death of the larvae and as a consequence, increase in the percentage of larvae death.

This study has proven the anthelmintic properties of CPE and thus it can be considered as an alternative to the commercialized anthelmintic.

6.0 CONCLUSION AND RECOMMENDATIONS

In conclusion, the findings from this study showed that CPE has significant anthelmintic activities by causing death of the L3 of strongyles from sheep. In addition, the CPE at 12.5 mg/ml induced the highest L3 mortality rate in 48h. Thus, CPE has a high potential as an alternative non-chemical method for control of helminthiasis in ruminants in the future. However, *in vivo* studies are required to confirm the anthelmintic activities in target host species. More studies should also be conducted to determine the mechanism of action of the bioactive compounds and the metabolites in the papaya leaves that brings about the anthelmintic properties.

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8.0 APPENDICES

8.1 Flow Chart of Papaya Leaf Processing

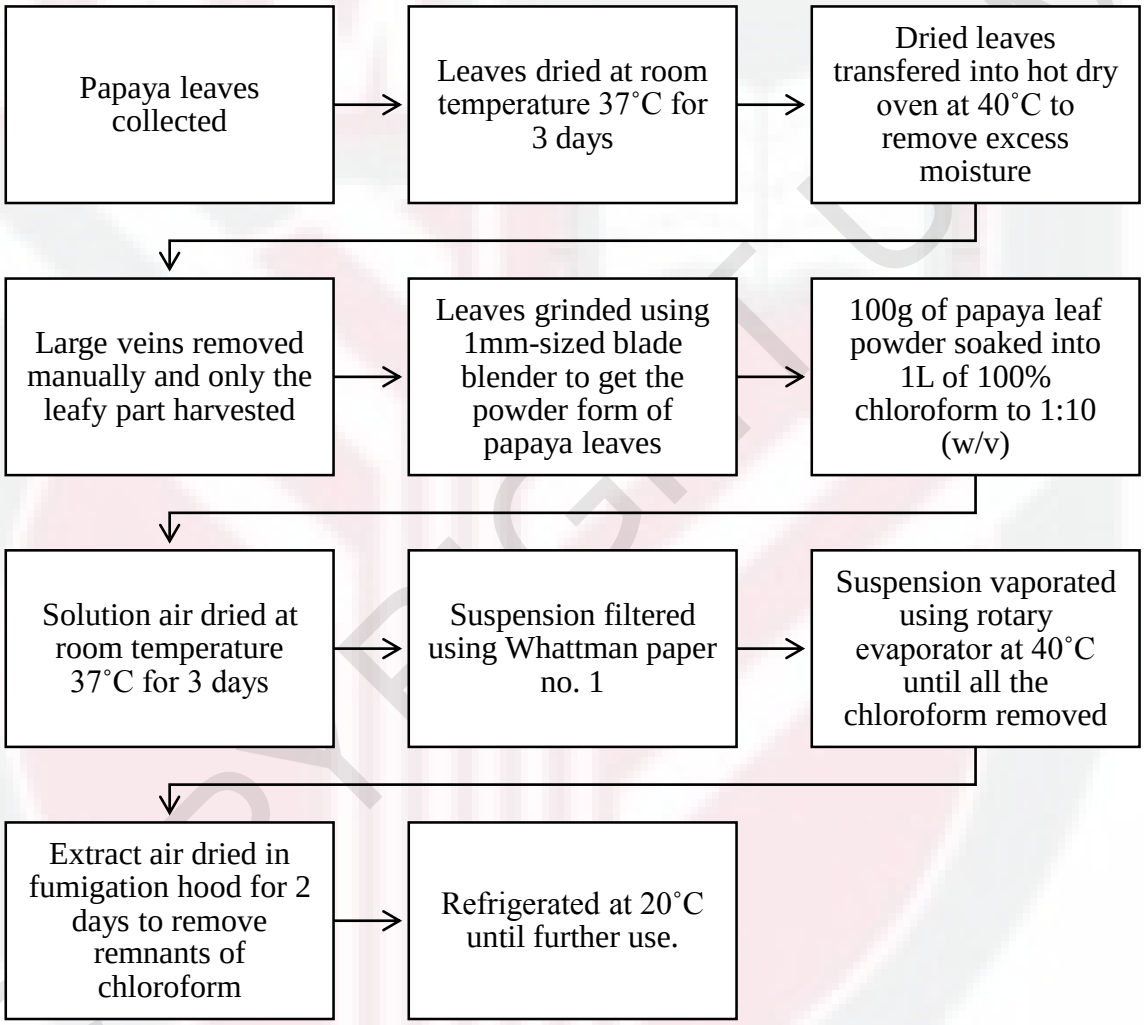

Figure 8.1.1 Flow Chart of the Papaya Leaves Processing

8.2 Faecal Processing and culture technique

Figure 8.2.1 Faecal culture technique

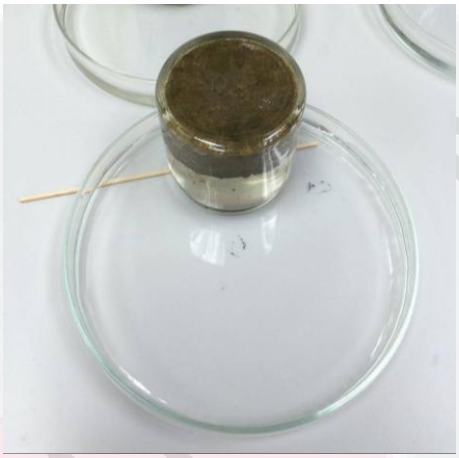

Figure 8.2.2 Modified Baermann Technique

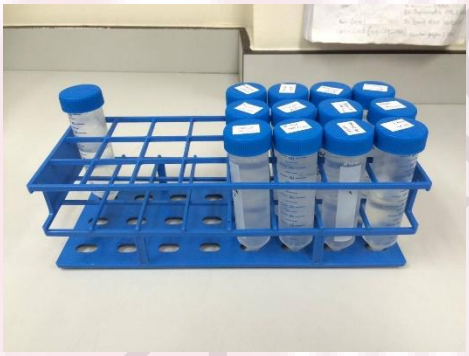

Figure 8.2.3 Stock solution containing L3 of strongyle

8.3 Papaya Leaf Processing

Figure 8.3.1 Papaya leaves dried at room temperature 37˚C for 3 days

Figure 8.3.2 Hot dry oven to keep the leaves untill the leaves crisp and the weight even

Figure 8.3.3 Powder form of papaya leaves

Figure 8.3.4 Papaya leaf powder soaked in chloroform

Figure 8.3.5 Suspension filtered using Whatmann paper no. 1

Figure 8.3.6 Filtrate evaporated using Rotary Evaporator at 40˚C

8.4 Assessment of the Anthelmintic Activity

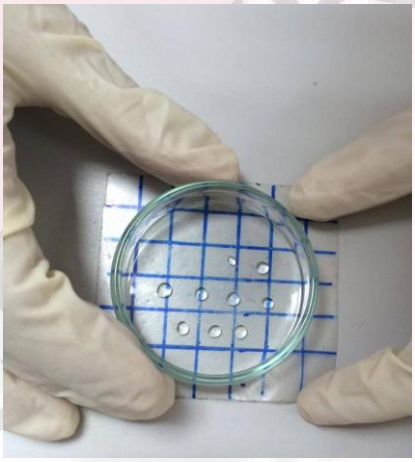

Figure 8.4.1 100 larvae per petri dish was counted using a grid

Figure 8.4.2 CPE at different concentration was placed and death of larvae was monitored using Stereo microscope

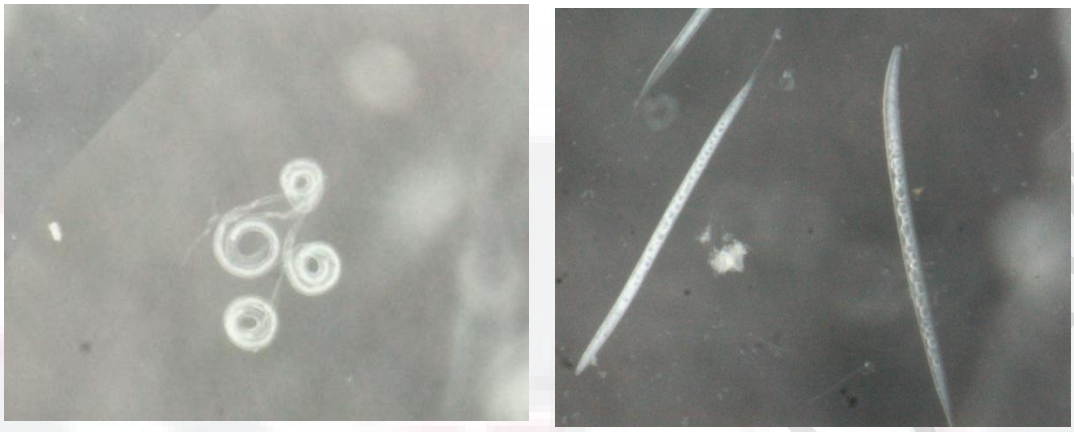

Figure 8.4.3 Morphology of L3 observed under stereo microscope on the right is alive larvae with coiled morphology. On the left, dead larvae with straightened morphology