



UNIVERSITI PUTRA MALAYSIA

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

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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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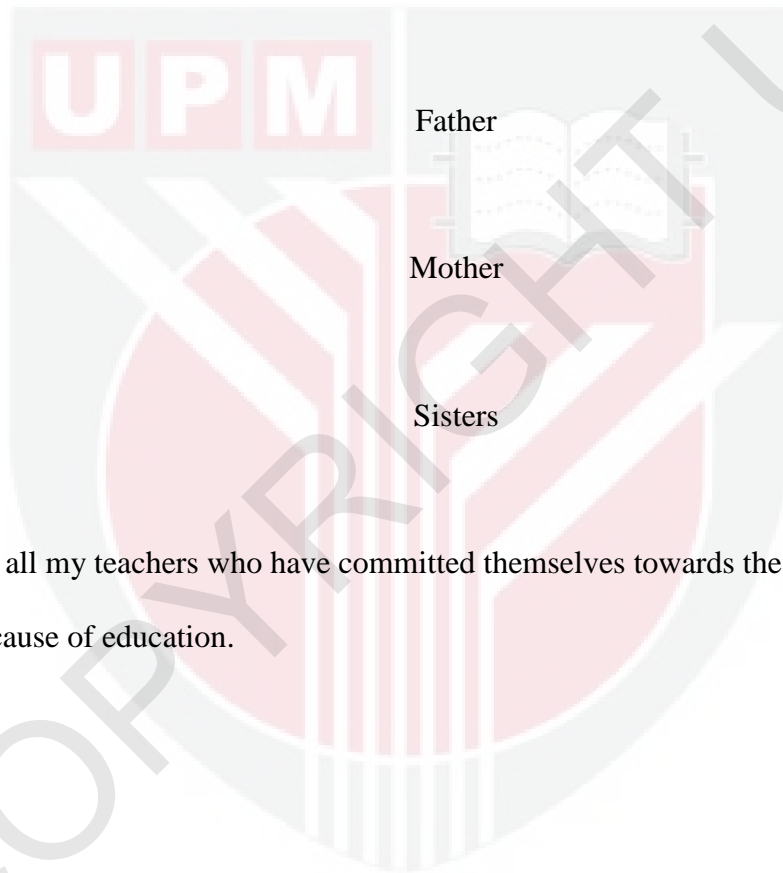
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DEDICATIONS

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

To my family,



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

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CONTENTS

TITLE	i
CERTIFICATION	ii
DEDICATIONS	iii
ACKNOWLEDGEMENTS	iv
CONTENTS.....	v
LIST OF TABLES	vi
LIST OF PLATES	vii
LIST OF ABBREVIATIONS	viii
ABSTRAK	ix
ABSTRACT	xi
1.0 INTRODUCTION.....	1
1.1 Background	1
1.2 Justification	3
1.3 Objectives.....	3
1.4 Hypothesis.....	3
2.0 LITERATURE REVIEW.....	4
2.1 Strongylida in Sheep	4
2.2 <i>Carica papaya</i> (papaya).....	6
2.3 Anthelmintic Usage.....	7
3.0 MATERIALS AND METHODS	9
3.1 Faecal sample, culture and harvesting L3	9
3.2 Collection and processing of plant material	9
3.3 Preparation of the chloroform extract (CPE)	10
3.4 Preparation of diluents and CPE.....	10
3.5 Experimental design	10
3.6 Assessment of the Anthelmintic Activity	11
3.7 Statistical Analysis	11
4.0 RESULTS	12
4.1 The <i>in vitro</i> anthelmintic effect of CPE on L3	12
5.0 DISCUSSION	15
6.0 CONCLUSION AND RECOMMENDATIONS	18
REFERENCES.....	19
8.0 APPENDICES.....	24

LIST OF TABLES

	Page
Table 1 The mean \pm 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)	12
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)	12

LIST OF PLATES

		Page
Figure 1	General Strongyle Life Cycle	5
Figure 2	Locations of Strongyle Found in Sheep	5
Figure 3	Effect of Papaya Leaves Chloroform Extract (CPE) on the L3 Mortality (%) over time (hours)	14
Figure 8.1.1	Flow Chart of the Papaya Leaf Processing	24
Figure 8.2.1	Faecal culture technique	25
Figure 8.2.2	Modified Baermann Technique	25
Figure 8.2.3	Stock solution containing L3 of strongyle	25
Figure 8.3.1	Papaya leaves dried at room temperature 37°c for 3 days	26
Figure 8.3.2	Hot dry oven	26
Figure 8.3.3	Powder form of papaya leaves	26
Figure 8.3.4	Papaya leaf powder soaked in chloroform	26
Figure 8.3.5	Suspension filtered using Whatmann paper no. 1	27
Figure 8.3.6	Filtrate evaporated using Rotary Evaporator at 40°C	27
Figure 8.4.1	100 larvae per petri dish was counted using grid	27
Figure 8.4.2	CPE at different concentration was placed and death of larvae was monitored using Stereo microscope	27
Figure 8.4.3	Morphology of L3	28

LIST OF ABBREVIATIONS

%	Percent
µL	Microlitre
df	Degree of freedom
g	Grams
kg	Kilograms
mg	Miligrams
mg/ml	Milligram per millilitres
ml	Millilitres
CPE	<i>Carica papaya</i> extract
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 KLOOROFOM DAUN
BETIK (*CARICA PAPAYA*) KE ATAS LARVA STRONGIL PERINGKAT
KETIGA DARIPADA BIRI-BIRI**

Oleh

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2016

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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

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2016

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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 petri 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.

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